

AD-A187 076 THE USE OF ATP-MGCL2 IN THE TREATMENT OF INJURY AND SHOCK(U) YALE UNIV NEW HAVENCONN DEPT OF SURGERY A E BAUE ET AL 31 JUL 86 DAMD17-81-C-1170

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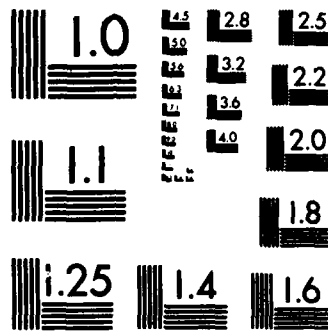
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THE USE OF ATP-MgCl<sub>2</sub> IN THE TREATMENT  
OF INJURY AND SHOCK

Annual/Final Report

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IRSHAD H. CHAUDRY, Ph.D.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The purpose of our study was to determine how ATP-MgCl <sub>2</sub> might be used, what the potential problems with ATP-MgCl <sub>2</sub> might be and to develop all the necessary background information in order to initiate clinical trials. In normal as well as hypovolemic awake dogs, cardiac output can be increased significantly by infusion of ATP-MgCl <sub>2</sub> intravenously at rates of 0.5-2.5 mg/Kg/min. Although higher doses of ATP-MgCl <sub>2</sub> may have detrimental hemodynamic effects, such effects are immediately reversible by ceasing ATP-MgCl <sub>2</sub> infusion. Moreover, the deleterious hemodynamic effects of very high doses of ATP-MgCl <sub>2</sub> can be markedly ameliorated.		

by atropine. Infusion of ATP-MgCl<sub>2</sub> did not adversely affect hepatic or renal function, myocardium or blood chemistry shortly after infusion or even after a prolonged period of time. Infusion of even massive doses of ATP-MgCl<sub>2</sub> have no ill effects on the survival of dogs. ATP-MgCl<sub>2</sub> can be prepared sterile and pyrogen free and the solution is stable at room temperatures for at least six months if stored in sterile, sealed ampules. The response of the primates to ATP-MgCl<sub>2</sub> infusion was somewhat similar to that of dogs. There were no long-term adverse neurological effects of ATP-MgCl<sub>2</sub> infusion in primates.

In conscious rats and primates bolus infusion of the entire efficacious dose of ATP-MgCl<sub>2</sub> transiently decreased mean blood pressure but there were no long-term side effects and there were no mortalities. Moreover, daily administration of the entire efficacious dose of ATP-MgCl<sub>2</sub> for a period of three months in rats did not produce any mortality or adverse side effects. ATP-MgCl<sub>2</sub> was approved for clinical studies by the Food and Drug Administration and the U.S. Army's and our own Human Investigation Committee. In accordance with a protocol approved by the above committees, five healthy adult male volunteers received an intravenous infusion of ATP-MgCl<sub>2</sub> (0.1-0.4 mg/kg/min) on four separate occasions. The total dose infused was 3, 6, 10 and 30 mg/kg (n=20 studies). The results indicated that cardiac output increased by 76% from control values with ATP-MgCl<sub>2</sub> infusion and stroke volume index increased by 14%, however, the mean blood pressure did not change significantly over the entire range of infusion rates. These results demonstrate the safety of ATP-MgCl<sub>2</sub> administration in humans and indicate that infusion of this agent at certain doses increases cardiac output significantly without producing hypotension. These data suggest a potentially beneficial role of ATP-MgCl<sub>2</sub> in the treatment of low flow states in humans.

Our results also indicated that infusion of ATP-MgCl<sub>2</sub> in dogs, even during hypotension increases coronary flow and cardiac output while decreasing myocardial oxygen consumption. This combination of increased cardiac output with decreased myocardial O<sub>2</sub> consumption supports a role for therapeutic use of ATP-MgCl<sub>2</sub> during low flow states and with coronary insufficiency. Our results also indicated that ATP-MgCl<sub>2</sub> infusion decreases total body oxygen consumption despite a concomitant increase in cardiac output and oxygen delivery.

We also submitted the protocol of our Phase II studies of ATP-MgCl<sub>2</sub> to our Human Investigation Committee as well as the Army's Human Investigation Committee and our protocol was approved. We evaluated the safety of ATP-MgCl<sub>2</sub> administration in six patients with coronary artery disease. ATP-MgCl<sub>2</sub> infusion was carried out into the left main coronary artery of such patients during routine diagnostic coronary angiography procedures. There were no measurable effects of ATP-MgCl<sub>2</sub> infusion on blood pressure, heart rate or cardiac output by administering 0.01-0.037 mg/kg/min ATP-MgCl<sub>2</sub>. There was no visible contractile abnormality during the ATP-MgCl<sub>2</sub> infusion. Our results demonstrated a 65% increase in coronary sinus blood flow with a concomitant 27% reduction in myocardial oxygen consumption, indicating that ATP-MgCl<sub>2</sub> is a demand-independent coronary vasodilator. The reduction in myocardial O<sub>2</sub> consumption in the absence of changes in the measured determinants of myocardial O<sub>2</sub> demand suggests a possible O<sub>2</sub> sparing effect of ATP-MgCl<sub>2</sub>. These results therefore indicate that ATP-MgCl<sub>2</sub> can be infused safely in patients with coronary artery disease and that infusion of ATP-MgCl<sub>2</sub> up to 0.037 mg/kg/min does not produce any bradycardia or decrease in blood pressure but does increase coronary sinus blood flow and decreases myocardial O<sub>2</sub> consumption. Thus, ATP-MgCl<sub>2</sub> shows favorable characteristics for potential application in patients with coronary artery disease.

## SUMMARY

The purpose of our studies was to determine how ATP-MgCl<sub>2</sub> might be used, what the potential problems with ATP-MgCl<sub>2</sub> might be and to develop all the necessary background information in order to initiate clinical trials of this agent. Our studies have shown that in normal alert dogs, cardiac output can be increased significantly by infusion of ATP-MgCl<sub>2</sub> intravenously at rates of 0.5-2.5 mg/Kg/min.. Although higher doses of ATP-MgCl<sub>2</sub> may have detrimental hemodynamic effects, such effects are immediately reversible by ceasing ATP-MgCl<sub>2</sub> infusion. Moreover, the deleterious hemodynamic effects (bradycardia and decreased cardiac output) of very high doses of ATP-MgCl<sub>2</sub> can be markedly ameliorated by administration of atropine. The results also indicate that the hemodynamic effects observed are caused by ATP-MgCl<sub>2</sub> and are not dependant upon the presence of vanadate. ATP-MgCl<sub>2</sub> infusion can produce profound peripheral vasodilatory effects while actually increasing cardiac output even in awake hypovolemic dogs. This suggests that ATP-MgCl<sub>2</sub> may prove beneficial in improving tissue perfusion in low output states. Infusion of ATP-MgCl<sub>2</sub> did not adversely affect platelet counts, white blood cell counts and differential or serum magnesium levels. Moreover, infusion of ATP-MgCl<sub>2</sub> did not adversely affect hepatic or renal function or the myocardium either shortly after infusion or even after a prolonged period of time. Infusion of massive doses of ATP-MgCl<sub>2</sub> have no ill effects on the survival of animals. ATP-MgCl<sub>2</sub> can be prepared sterile and pyrogen free and the solution is stable at room temperatures for prolonged periods of time if stored in sterile, sealed ampules.

The response of primates to ATP-MgCl<sub>2</sub> infusion was similar to that of dogs, however, the absolute response in cardiac output was different in the two species. Whereas continuous infusion of ATP-MgCl<sub>2</sub> in dogs increased cardiac output, reinfusion or "priming" the system with ATP-MgCl<sub>2</sub> was required in order to increase cardiac output in primates. Nonetheless, the fact remains that after "priming" the system with ATP-MgCl<sub>2</sub>, cardiac output can be increased up to 55% in conscious normovolemic as well as hypovolemic primates. Moreover, improvement of cardiac output can be obtained with ATP-MgCl<sub>2</sub> without exacerbation of hypotension in hypovolemic primates. The results also suggest that Cynomolgus monkeys tend to be extremely tolerant of hemorrhage. Despite various attempts, we were unable to establish a reproducible model of hemorrhagic shock in Cynomolgus monkeys. In view of that, we could not test the effects on ATP-MgCl<sub>2</sub> on survival of primates following hemorrhagic shock.

The neurological examination of conscious normal primates during ATP-MgCl<sub>2</sub> was also carried out. Infusion of low doses of ATP-MgCl<sub>2</sub> for seven minutes showed no change in EEG activity. Infusion of higher doses of ATP-MgCl<sub>2</sub>, however, decreased the voltage and slow waves appeared symmetrically as mean arterial blood pressure began to fall to a level of 45 mmHg. When the infusion was stopped, the EEG pattern remained slow for some time, however, the activity picked up when the mean arterial blood pressure reached 90mmHg. At the end of the experiment, the animals were alert and the EEG was back to baseline Beta-activity. The behavioral changes noted during infusion of high doses of ATP-MgCl<sub>2</sub> were consistent with that of suppression of level of consciousness and drowsiness when the mean blood pressure was within the range of 20-25mmHg.



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The animals, however, remained symmetrical when stimulated with a normal grasp and pupils remained reactive. The levels of responsiveness increased with the return of blood pressure to a mean of 90mmHg and this was concomitant with the return to a normal EEG baseline activity.

Our studies also showed that even rapid infusion of the entire efficacious dose of ATP-MgCl<sub>2</sub> into rats did not produce any mortality. Moreover, rapid infusion of ATP-MgCl<sub>2</sub> daily for a period of 5 days did not produce any mortality and there were no apparent long-term side effects of ATP-MgCl<sub>2</sub> infusion in these animals in comparison to rats receiving saline for the same period of time. The results also indicated that even if a total of eight times the efficacious dose of ATP-MgCl<sub>2</sub> was infused over a very short period of time in conscious rats, none of the animals died or showed any long term side effects. Our results also showed that intraperitoneal injections of ATP-MgCl<sub>2</sub> daily in rats for a period of three months also did not produce any mortality and there were no apparent side effects due to such injections.

The response of the primates to bolus infusions of ATP-MgCl<sub>2</sub> was similar to that of rats. Although the decrease in blood pressure with bolus ATP-MgCl<sub>2</sub> infusion was more in the primates than in the rats, there were no mortalities due to such bolus infusions of ATP-MgCl<sub>2</sub>. Thus, both species of animals, i.e., rats and primates, tolerated the bolus and repeated infusions of ATP-MgCl<sub>2</sub> and there were no apparent side effects.

We also submitted the protocol of our studies to our Human Investigation Committee for their approval of ATP-MgCl<sub>2</sub> for the phase I studies. In addition, the protocol was submitted to the Army's Human Investigation Committee and it was approved by both our as well as the Army's Human Investigation Committee. We also submitted an application to the Food and Drug Administration seeking permission to use ATP-MgCl<sub>2</sub> in normal human volunteers. The investigative new drug application was approved by the Food and Drug Administration.

Our objectives were to also determine the safety and hemodynamic response of ATP-MgCl<sub>2</sub> infusion in normal awake human volunteers. In accordance with a protocol approved by the Human Investigation Committee, five healthy adult male volunteers received an intravenous infusion of ATP-MgCl<sub>2</sub> (0.1-0.4mg/kg/min) on four separate occasions. The total dose infused was 3, 6, 10 and 30mg/kg (n=20 studies). Hemodynamic measurements were made at end exhalation in the supine position and included heart rate and systolic, diastolic and mean blood pressure. Continuous electrocardiographic monitoring of lead II was performed. Cardiac output was determined by injection of indocyanine green and measured by the principle of earpiece densitometry. Measurements were made prior to infusion, at 5 minute intervals during infusion and following termination of the infusion. Blood samples were obtained for determination of hemoglobin, sodium, potassium and glucose. Stroke volume index (SVI) and total systemic resistance (TSR) were derived from standard formulae. The results indicated that cardiac output increased by 76% from control values ( $p < 0.0001$ ) with ATP-MgCl<sub>2</sub> infusion. This increase in cardiac output correlated positively with ATP-MgCl<sub>2</sub> infusion rate ( $r=0.75$ ,  $p < 0.001$ ). This was paralleled by a 45% increase in heart rate ( $p < 0.0001$ ). SVI increased by 14% ( $p < 0.005$ ), however, the mean blood pressure did not change significantly over the entire range of

infusion rates. TSR decreased 56% at the highest rate of ATP-MgCl<sub>2</sub> infusion. A mean infusion rate of  $0.32 \pm 0.02$  mg/kg/min was associated with maximal increases in heart rate (52%) and cardiac output (119%) without affecting mean blood pressure. Hemodynamic effects were poorly correlated with total dose of ATP-MgCl<sub>2</sub> ( $r=0.20$ ).

All hemodynamic changes returned to normal within 2 minutes after the ATP-MgCl<sub>2</sub> infusion was discontinued ( $p=NS$ ). Sodium, potassium, hemoglobin and glucose levels did not change during or after ATP-MgCl<sub>2</sub> administration. All subjects experienced transient mild nausea at infusion rates greater than 0.3 mg/kg/min. There were no delayed side effects.

ATP-MgCl<sub>2</sub> is a potent vasodilator. As demonstrated in this study, the increase in cardiac output offset the decrease in total systemic resistance. Thus, blood pressure (MBP) was maintained. Furthermore, the increase in SVI suggests a mild inotropic effect. These findings suggest that the increase in heart rate and cardiac output may depend upon an intact sympathetic nervous system. In addition, the pharmacologic profile of vasodilatation, augmentation of cardiac output, maintenance of blood pressure and mild positive inotropy coupled with beneficial effects on cell function and survival in animal studies suggest potential clinical applications of ATP-MgCl<sub>2</sub> in patients with low flow states or organ ischemia. In conclusion, data from our studies suggest a potentially beneficial role of ATP-MgCl<sub>2</sub> in the treatment of low flow states and confirms the safety of ATP-MgCl<sub>2</sub> in humans.

In the Phase I studies of ATP-MgCl<sub>2</sub> in normal volunteers, most volunteers demonstrated tachycardia during infusion of this agent. It could thus be argued that tachycardia which was observed with ATP-MgCl<sub>2</sub> infusion and its attendant effects on myocardial oxygen demand may well set the stage for exacerbation of any deleterious mismatch between myocardial oxygen demand and myocardial oxygen supply. To study this, additional experiments were conducted in dogs. Our results indicated that even during hypotension ATP-MgCl<sub>2</sub> increases coronary flow and cardiac output while decreasing myocardial O<sub>2</sub> consumption. Although both ATP-MgCl<sub>2</sub> and nitroprusside reduced myocardial O<sub>2</sub> consumption through after-load reduction (decreased work), ATP-MgCl<sub>2</sub> but not nitroprusside decreases myocardial O<sub>2</sub> consumption for any given workload. This indicates an additional metabolic effect of ATP-MgCl<sub>2</sub>. This combination of increased cardiac output with decreased myocardial O<sub>2</sub> consumption supports a role for therapeutic use of ATP-MgCl<sub>2</sub> during low flow states and with coronary insufficiency. These results therefore indicate that ATP-MgCl<sub>2</sub> administration does not cause any deleterious mismatch between myocardial oxygen demand and myocardial oxygen supply.

We have also determined whether ATP-MgCl<sub>2</sub> administration has any deleterious effects on whole body oxygen consumption in dogs. The results of such experiments indicated that ATP-MgCl<sub>2</sub> infusion decreases total body oxygen consumption despite a concomitant increase in cardiac output and oxygen delivery. This could well be a metabolic effect of infused ATP-MgCl<sub>2</sub>.

We also submitted the protocol of our Phase II studies of ATP-MgCl<sub>2</sub> to our Human Investigation Committee and they approved the protocol. In addition, the protocol was submitted to the Army's Human Investigation Committee and it was



approved by the U.S. Army's Human Investigation Committee. We then attempted to determine the safety of ATP-MgCl<sub>2</sub> administration in six patients with coronary artery disease. ATP-MgCl<sub>2</sub> was infused into the left main coronary artery of these patients during a routine diagnostic coronary angiography. There was no measurable effect on blood pressure, heart rate or cardiac output which all remained at baseline levels during the infusion of 0.01-0.037ml/kg/min ATP-MgCl<sub>2</sub>. There were no visible contractile abnormalities during the ATP-MgCl<sub>2</sub> as monitored by two-dimensional echocardiography. In some patients coronary sinus catheters were inserted and the effects of ATP-MgCl<sub>2</sub> infusion on coronary sinus blood flow and myocardial oxygen consumption was investigated. The results demonstrated a 65% increase in coronary sinus blood flow with a concomitant 27% reduction in myocardial oxygen consumption, indicating that ATP-MgCl<sub>2</sub> is a demand-independent coronary vasodilator. The reduction in myocardial oxygen consumption in the absence of changes in the measured determinants of myocardial oxygen demand suggests a potential oxygen sparing effect of ATP-MgCl<sub>2</sub>. These results therefore indicate that ATP-MgCl<sub>2</sub> can be infused safely in patients with coronary artery disease and that infusion of ATP-MgCl<sub>2</sub> up to 0.037ml/kg/min does not produce any bradycardia or decrease in blood pressure but does increase coronary sinus blood flow and decreases myocardial oxygen consumption. Thus, ATP-MgCl<sub>2</sub> shows favorable characteristics for potential applications in patients with coronary artery disease.

#### FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

The clinical studies described in this report were conducted after we obtained the IND for ATP-MgCl<sub>2</sub> from the Food and Drug Administration and following the approval of our protocol from the Army's Human Investigation Committee, our local Human Investigation Committee and from the Food and Drug Administration.

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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### Body of Report

Work completed during the period of September 15, 1981 through September 30, 1985 can best be summarized by citing the publications from our laboratory supported by the above contract:

1. Chaudry, I.H., Clemens, M.G. and Baue, A.E.: Alterations in cell function with ischemia and shock and their correction. *Arch. Surg.* 116:1309-1317, 1981.
2. Chaudry, I.H., Schleck, S., Kovacs, K.F. and Baue, A.E.: Effect of prolonged starvation on reticuloendothelial function and survival following trauma. *J. Trauma* 21:604-611, 1981.
3. Chaudry, K.H. and Baue, A.E.: Overview of hemorrhagic shock. In: Cell Injury and Shock, Anoxia and Ischemia (ed. Cowley, R.A. and Trump, B.F.) Williams and Wilking, Baltimore, pp203-219, 1982.
4. Baue, A.E., Clemens, M.G. and Chaudry, I.H.: Hemodynamic effects of ATP-MgCl<sub>2</sub> infusion in conscious, normovolemic (NV) and hypovolemic (HV) dogs. *Fed. Proc.* 41(#4):1131, 1982.
5. Clemens, M.G., McDermott, P.H., Chaudry, I.H. and Baue, A.E.: Hemodynamic effects of ATP-MgCl<sub>2</sub> infusion in conscious, normovolemic and hypovolemic primates. *Circ. Shock* 9:185, 1982.
6. Chaudry, I.H.: Preparation of ATP-MgCl<sub>2</sub> and precautions for its use in the study and treatment of shock and ischemia. *Am. J. Physiol.* 242:R604-605, 1982.
7. Chaudry, I.H. and Baue, A.E.: Energy problems during shock and their correction with adenosine triphosphate-magnesium chloride. In: The Role of Chemical Mediators in the Hemodynamic, Pulmonary and Metabolic Failure in the Acutely-Ill and Injured Patients. McConn R. (ed), Raven Press, New York, pp 361-376, 1982.
8. Chaudry IH, Baue AE: Pharmacologic intervention in shock. *Proc Int Burn Res Conference*, January 19-21, 1983, San Antonio, Texas, pp 41-46.
9. Chaudry IH, Baue AE: Electrolyte and water metabolism in shock and trauma. In: Altura BM, Lefer AM, Schurer W (eds), The Handbook of Shock and Trauma, Vol 1, New York, Raven Press, pp 227-240, 1983.
10. Chaudry IH: Cellular mechanisms in shock and ischemia and their correction. *Am J Physiol* 245:R117-R134, 1983.
11. Chaudry IH, Keefer R, Barash P, Clemens MG, Kopf G, Baue AE: ATP-MgCl<sub>2</sub> (ATP) infusion in man: Increased cardiac output without adverse systemic hemodynamic effects. *Surg Forum* 35:14-16, 1984.
12. Keefer JR, Chaudry IH, Barash PG, Clemens MG, Baue AE: ATP-MgCl<sub>2</sub>: Safety and hemodynamic responses in humans. *Anesthesiology* 61:A119, 1984.

13. Chaudry IH: Cellular energetics and ATP-MgCl<sub>2</sub> therapy in sepsis. Am J Emerg Med 2:38-44, 1984.
14. Krinsley J, Chaudry IH, Loke J, Snyder P, Virgulto J, Geha A, Baue AE: "Effect of adenosine triphosphate-magnesium chloride infusion on oxygen consumption and hemodynamics in anesthetized dogs". Am Rev Resp Dis 131(4):A310, 1985.
15. Clemers MG, Chaudry IH, Baue AE: "Increased coronary flow and myocardial efficiency with systemic infusion of ATP-MgCl<sub>2</sub>". Surg Forum (In Press).
16. Wohlgelernter D, Jaffe C, Cleman M, Young L, Clemens M, Chaudry IH: "Effects of ATP-MgCl<sub>2</sub> on coronary blood flow and myocardial oxygen consumption". Circulation (In Press).

Reprints of the articles published and those in press are included for review. We have also participated in a number of programs in which the work supported by this contract has been presented. These include participation and presentation of our work at the Annual American College of Surgeons meeting, American Association for the Surgery of Trauma meeting, Shock Society Meeting, International Burn Research Conference in San Antonio, Texas, the Federation Meeting and various other lectures at regional and local programs on shock and circulatory failure.

The principal findings of the above contract will now be summarized.

1. Hemodynamic Responses to ATP-MgCl<sub>2</sub> Infusion Via the Right Atrium in Normal Alert Dogs.

Infusion of ATP-MgCl<sub>2</sub> in conscious alert dogs at rates of up to 2.5 mg/kg/min consistently produces vasodilation, increased pulse pressure, increased cardiac output and a tachycardia. At these doses, myocardial contractility as assessed by left ventricular dP/dT showed little if any, change. At higher doses, however, a bradycardia and variable decrease in dP/dT was observed. At an infusion rate of 5.0mg/kg/min, cardiac output was still increased by ATP-MgCl<sub>2</sub> infusion. Infusion of 10mg/kg/min, however, consistently produced a fall in cardiac output along with bradycardia and decreased contractility which was rapidly reversed on termination of infusion. At all concentrations of ATP-MgCl<sub>2</sub> above threshold, pulse pressure was markedly increased apparently as a result of decreased peripheral resistance. These results indicate that infusion of ATP-MgCl<sub>2</sub> at rates of 0.5-2.5 mg/kg/min is effective in increasing cardiac output along with marked peripheral vasodilation. Thus, while higher doses may have detrimental hemodynamic effects, these effects are immediately reversible by ceasing ATP-MgCl<sub>2</sub> infusion.

2. Hemodynamic (Dose-Response) Effect of ATP-MgCl<sub>2</sub> Infusion in Hypovolemic Awake Dogs.

The hemodynamic responses of alert hypovolemic dogs (bled to mean arterial pressure of about 80mmHg) to ATP-MgCl<sub>2</sub> were similar to those of normal dogs. Infusion of ATP-MgCl<sub>2</sub> at rates below 2.5mg/kg/min resulted in peripheral

vasodilation, increase in pulse pressure and increase in cardiac output. At higher doses, the peripheral vasodilatory response was still observed, but cardiac output tended to fall. The increase in cardiac output in the hypovolemic dogs was, however, not as marked as that seen in normal dogs (about 35% peak increase in hypovolemic dogs compared to greater than 100% increase in some normovolemic dogs). Furthermore, while the normovolemic dogs responded with a tachycardia at lower doses of ATP-MgCl<sub>2</sub>, the hypovolemic dogs showed a bradycardia with all effective doses of ATP-MgCl<sub>2</sub>. The observations that ATP-MgCl<sub>2</sub> infusion can produce profound peripheral vasodilatory effects while actually increasing cardiac output even in hypovolemic dogs suggest that ATP-MgCl<sub>2</sub> may prove beneficial in improving tissue perfusion in low output states.

### 3. Effect of Vanadate Contamination of ATP From Equine Muscle.

The ATP that we have used for all experiments demonstrating the salutary effects of ATP-MgCl<sub>2</sub> has been Sigma's equine muscle ATP. This has been considered to be the highest purity ATP available. Recent reports, however, have demonstrated that a major contaminant of equine muscle ATP is vanadate. The vanadate ion has now been shown to have a variety of physiological effects, including inhibition of Na<sup>+</sup>-K<sup>+</sup> ATPase. To test the possibility that the hemodynamic effects seen during ATP-Cl<sub>2</sub> infusion are dependent upon the presence of vanadate, several experiments were performed using Sigma's new vanadium-free ATP from equine muscle. The hemodynamic responses to the vanadium-free ATP were indistinguishable from the responses to the vanadium-containing ATP. These results therefore demonstrate that the hemodynamic effects observed are caused by ATP-MgCl<sub>2</sub> and are not dependent upon the presence of vanadate.

### 4. Effect of ATP-MgCl<sub>2</sub> Infusion on Hepatic and Renal Function.

We have conducted extensive studies dealing with the effects of ATP-MgCl<sub>2</sub> infusion on serum GOT, GPT, alkaline phosphatase and creatinine levels before and 5 days following ATP-MgCl<sub>2</sub> infusion. Our results indicated that there was no significant effect of ATP-Cl<sub>2</sub> on the levels of any of the above parameters. Thus, we can conclude that infusion of ATP-MgCl<sub>2</sub> did not adversely affect hepatic or renal function either shortly after infusion or even after a prolonged period of time.

### 5. Effect of ATP-MgCl<sub>2</sub> Infusion on CPK and LDH Enzymes.

We have also studied the effects of ATP-MgCl<sub>2</sub> infusion on CPK and CPK isoenzymes as well as LDH and its isoenzymes following infusion of ATP-MgCl<sub>2</sub>. There was no significant effect of ATP-MgCl<sub>2</sub> on the level of any of the above enzymes indicating that there was no deleterious effects of ATP-MgCl<sub>2</sub> infusion on the myocardium as well.

### 6. Effect of ATP-MgCl<sub>2</sub> on Serum Magnesium Levels.

Serum magnesium levels were also measured with and without ATP-MgCl<sub>2</sub> infusion in a number of dogs. The results indicated that following infusion of ATP-MgCl<sub>2</sub>, the serum magnesium levels increased approximately 25%. However, 5

days following ATP-MgCl<sub>2</sub> infusion, the levels restore to normal. It is not surprising that the serum magnesium levels increase following infusion of ATP-MgCl<sub>2</sub> since MgCl<sub>2</sub> along with ATP was infused. Samples for magnesium levels were not taken between days 1 and 5 post ATP-MgCl<sub>2</sub> infusion. It is, however, possible that magnesium levels return to normal prior to day 5. The return towards normal magnesium levels 5 days following ATP-MgCl<sub>2</sub> infusion, however, indicates that the short term increase in serum magnesium levels is not of any major concern.

#### 7. Effect of ATP-MgCl<sub>2</sub> on Platelet Counts, White Blood Counts and Differential.

We have conducted additional studies on WBC and differential, platelets, hematocrit and hemoglobin measurements prior to ATP-MgCl<sub>2</sub> infusion and at various intervals during and 5 days following infusion of ATP-MgCl<sub>2</sub>. There was no significant effect of ATP-MgCl<sub>2</sub> on any of the above parameters. WBC counts were slightly elevated, however, this was due to incisions made for catheters rather than to ATP-MgCl<sub>2</sub> infusion. In addition, WBC counts returned to normal within 5 days post-ATP-MgCl<sub>2</sub> infusion.

#### 8. Safety of Massive ATP-MgCl<sub>2</sub> Infusion in Dogs.

We have now carried out additional studies in which we infused ATP-MgCl<sub>2</sub> in awake normovolemic and hypovolemic dogs at a concentration which is 3 times the required efficacious dose of ATP-MgCl<sub>2</sub>. Our results indicated that none of the animals died as a result of this either immediately or even after 3 months (at which time the observations were terminated). These experiments therefore clearly indicate that infusion of even massive doses of ATP-MgCl<sub>2</sub> have no ill effect on the survival of animals. In addition, the animals did not exhibit any altered behavior during or following ATP-MgCl<sub>2</sub> infusion.

#### 9. The Effect of Atropine on ATP-MgCl<sub>2</sub> Induced Bradycardia.

One of the major problems associated with administration of very large doses of ATP-MgCl<sub>2</sub> is a paradoxical bradycardia in the face of severe hypotension. Since these doses also produced increased salivation, we administered atropine to three dogs to test whether the bradycardia was produced by increased parasympathetic activity. While ATP-MgCl<sub>2</sub> infusion at a rate of 2.5mg/kg/min consistently decreased heart rate by as much as 34% in the control state, heart rate increased in response to 2.5 or 5.0mg/kg/min after the administration of atropine. Thus, blockade of parasympathetic transmission reversed the heart rate response to one associated with concomitant increases in cardiac output. These results indicate that the deleterious hemodynamic effects of very high doses of ATP-MgCl<sub>2</sub> can be markedly ameliorated by the administration of atropine.

#### 10. Stability of ATP-MgCl<sub>2</sub> Complex.

Our results indicated that if ATP-MgCl<sub>2</sub> solutions were stored at room temperature, there was some bacterial growth within a month of storage and the ATP contents of the solution decreased by approximately 25%. There was no significant decrease in ATP contents of the solutions if such solutions were

stored frozen at  $-70^{\circ}\text{C}$ . In view of this, additional studies were conducted to determine whether bacterial growth and therefore the breakdown of ATP could be prevented during storage of ATP-MgCl<sub>2</sub> solutions at room temperatures. The following solutions were prepared:

1. ATP in sterile water and pH adjusted to 7.4
2. ATP in phosphate buffer (pH 7.4)
3. ATP-MgCl<sub>2</sub> in sterile water and pH adjusted to 7.4
4. ATP-MgCl<sub>2</sub> in phosphate buffer (pH 7.4)

The sodium hydroxide solution used to adjust the pH was filtered through millipore filter prior to use. The ATP or ATP-MgCl<sub>2</sub> solution was passed through 0.22micromillipore filter prior to storage in microfuge tubes. Half of the microfuge tubes were stored at room temperature while the other half were stored at  $-70^{\circ}\text{C}$ .

There was no bacterial growth even after 6 months of storage of ATP-MgCl<sub>2</sub> at room temperature. After 6 months of storage, there was a slight decrease in the concentration of ATP solution which was stored at room temperature but was not found to be significantly different from the calculated concentration of ATP-MgCl<sub>2</sub>. Overall the results showed that more than 95% of ATP was detected enzymatically if the ATP-MgCl<sub>2</sub> solution was kept a room temperature or frozen for a period of at least six months. The presence or absence of phosphate buffer did not appear to make any difference in terms of the stability of ATP.

The above results lead us to conclude that no significant decrease in ATP contents of the ATP-MgCl<sub>2</sub> solution occur for at least six months if such solutions are kept sealed at room temperature.

#### 11. Pyrogenicity and Sterility Studies.

We have used different batches of ATP to test whether the ATP-MgCl<sub>2</sub> solution which we have been using contains any pyrogens. These studies were carried out by injecting ATP-MgCl<sub>2</sub> solutions in rabbits and recording any change in their body temperature. There was no significant increase in body temperature of rabbits following ATP-MgCl<sub>2</sub> administration suggesting the lack of pyrogens in the batches of ATP that we used. Thus, up to now we have tested five different batches of ATP and have not been able to find any pyrogens in them.

We have also conducted sterility studies on two additional batches of ATP and our results indicate that the ATP-MgCl<sub>2</sub> solutions were indeed sterile in the manner in which they were prepared. Thus, we can conclude that ATP and MgCl<sub>2</sub> solution could be prepared sterile and the batches of ATP that we used did not contain any pyrogens. Hence, ATP-MgCl<sub>2</sub> solution meets the requirement for administration in humans.

#### 12. Hemodynamic Response to ATP-MgCl<sub>2</sub> Infusion in Normovolemic Primates.

We experienced considerable delay in conducting hemodynamic studies in alert primates because of the quarantine regulations (two months) in our primate research facility. The delay was also caused because of the need for extensive



chair training of primates following release from quarantine. Chair training was essential since this allowed reliable study of alert animals. We have, however, been able to conduct four studies and have obtained some very promising data. In many ways, the response of the primates to ATP-MgCl<sub>2</sub> infusion is similar to that of the dogs in previous studies. There are, however, significant differences between the two species. In normovolemic primates we have been unable to show any cardiovascular effects with an ATP-MgCl<sub>2</sub> dose of 0.1mg/kg/min via the right atrium. At doses of 0.5mg/kg/min and higher they appear to be much more sensitive to the effects of ATP-MgCl<sub>2</sub> infusion than do the dogs. This is especially evident with response to the ATP-MgCl<sub>2</sub>-induced bradycardia that we have previously observed in dogs. This effect is evident at a dose of 0.5mg/kg/min (about 10% decrease in heart rate) and is profound at 2.5mg/kg/min (about 50% decrease). As a result of this paradoxical bradycardia, there is no significant increase in cardiac output at either 0.5 or 1.0mg/kg/min and at 2.5mg/kg/min cardiac output falls. Interestingly, if the mid-range doses (0.5 and 1.0mg/kg/min) are repeated after the monkey has been "primed" with a high-range dose (2.5mg/kg/min) significant increases in cardiac output are observed (up to 55% increase). This is not the result of reversing the heart rate response since there was no significant difference in heart rate response before vs after the high-range dose. At the present time the mechanism of this phenomenon is unclear. Nonetheless, the fact remains that by reinfusion of ATP-MgCl<sub>2</sub>, cardiac output can be increased up to 55% even in conscious normovolemic primates.

#### 13. Hemodynamic Response to ATP-MgCl<sub>2</sub> Infusion in Hypovolemic Primates.

We have conducted four experiments in which we infused ATP-MgCl<sub>2</sub> during hypovolemic conditions. Since the same monkeys were previously being used in normovolemic experiments, it was possible to make some interesting comparisons between the two conditions in the same animal. The most notable difference was the hypovolemic (mean arterial blood pressure 75-80mmHg) results in a greatly increased sensitivity to the effects of ATP-MgCl<sub>2</sub> infusion via the right atrium. While the monkeys tolerated infusion of ATP-MgCl<sub>2</sub> at a rate of 2.5mg/kg/min under normovolemic conditions, infusion of 1.0 mg/kg/min ATP-MgCl<sub>2</sub> produces severe hypotension (mean arterial blood pressure 15-20mmHg) when administered under hypovolemic conditions. The animals, however, recovered quite well from this severe hypotension once the infusion was terminated. Subsequently, infusion of 0.2mg/kg/min ATP-MgCl<sub>2</sub>, a dose which was previously ineffective, resulted in an increase in cardiac output of 55-60%. This dose, however, did not produce a fall in mean arterial blood pressure. Thus, after "priming" the system with ATP-MgCl<sub>2</sub>, improvement of cardiac output can be obtained with ATP-MgCl<sub>2</sub> without exacerbation of hypotension.

#### 14. Hemorrhagic Shock in Primates.

We initially attempted to produce a reproducible hemorrhage shock model of proper severity in primates for survival studies with ATP-MgCl<sub>2</sub> administration. Under nembutal anesthesia, blood was removed at a rate sufficient to reduce mean arterial pressure (MAP) to 40mmHg within 15 minutes (about 50% of the calculated blood volume which decreased cardiac output to less than 40% of control and central venous pressure to less than 1mmHg). Despite this severe cardiovascular insult, reinfusion of less than 10% of the shed blood

was required to maintain a MAP of 40mmHg for five hours. At that time the shed blood was returned and the catheters removed. The primate survived the procedure with no apparent ill effects. These results indicated that a more severe shock model was required for the planned survival studies.

The severity of the model was then increased by lowering the mean arterial pressure (MAP) to 30mmHg within 10 minutes and maintaining at  $30 \pm 5$ mmHg. Maximum bleedout occurred within 1 hour from the onset of hemorrhage. This required the removal of a total volume of blood equal to approximately 3% of the animal's body weight. The primates were maintained at a pressure of  $30 \pm 5$ mmHg for five hours. In addition, we have found that the animals could be humanely maintained without anesthesia after the bleedout has begun. This lack of anesthesia also increases the severity of the insult. Using this model, we have performed well regulated experiments in two male and two female primates. We did not include the third male primate in the study since we could not bleed that animal rapidly enough to drop his MAP to 30mmHg within ten minutes from the onset of hemorrhage. In the males, both animals used and included in the study required reinfusion of 30-40% of their shed blood to maintain a MAP of 30mmHg for five hours and were lethargic the day following the experiment. On the second day, one primate steadily improved and ultimately survived while the other became more lethargic and expired approximately 48 hours after the experiment. Thus, in the male primates this appeared initially to be a suitable model to study the effects of ATP-MgCl<sub>2</sub> on survival after shock. The females, however, appear to be more tolerant to hemorrhage. In contrast to the males, in both experiments with females, less than 10% of the total shed blood needed to be returned in order to maintain an MAP for 30mmHg for five hours. Both animals survived with no apparent ill effects.

Autopsy was performed on the primates which died. The findings indicated alterations in hepatic lobular architecture and renal cortical changes were also present. These hypovolemia-induced changes were deemed sufficient to cause death. Diffuse microfilarial infestations were also observed and may have increased this animal's susceptibility to hemorrhage. Thus, although the animal may have died of hemorrhagic shock, it is not known whether this animal was healthy to begin with.

In order to ascertain that we could produce a reproducible hemorrhagic shock model in primates, additional studies were conducted. It was possible to perform many more experiments than the original number of primates purchased because the procedure was repeated on survivors after several weeks recovery under the supervision of the of the veterinary staff in our primate facility. In these five experiments Cynomolgus monkeys were fasted for 24 hours prior to the experiment. The primates were then lightly anesthetized and a 20 gauge Jelco catheter was placed in the femoral artery. The animals were bled rapidly within 10 minutes to a mean arterial pressure of  $27 \pm 2$ mmHg and maintained at this level for ~~5~~ hours or until 40% of the shed blood had to be returned in order to maintain the above mean arterial pressure. The remaining shed blood was then returned following which the animals were returned to their cages. Survival was measured over a period of 5 days. Of these five primates, only 1 required more than 12% of its shed volume returned within the 5 hours time constraint of the experiment. This one primate required 40% of his shed blood to be returned within 2.5 hours following the initial hemorrhage and died within

24 hours. The four other primates, however, maintained their mean arterial blood pressure for the duration of the experiment with the return of only 4-12% of shed volume and recovered with no apparent ill effects resulting from the hemorrhage procedure.

From these results we are left to conclude that *Cynomolgus* monkeys tend to be extremely tolerant to hemorrhage and thus a reproducible hemorrhagic shock model cannot be established in this species. However, in a site visit, Dr. Ryan Neville of the Letterman Army Institute of Research informed us that Dr. Nelson Gurll from Iowa also had problems producing a reproducible hemorrhagic shock model in *Cynomolgus* monkeys but that he has now ironed out those problems. In view of the fact that we were having considerable problems in establishing a reproducible hemorrhagic shock model in *Cynomolgus* monkeys, we contacted Dr. David Reynolds in Iowa and made arrangements to visit his laboratory. Dr. Reynolds did set up a primate hemorrhagic shock model during our visit to their laboratory and the set up was as follows:

A *Cynomolgus* monkey (approximately 5 kg) was fasted for 24 hrs prior to the study. After ketamine anesthesia, the animal was intubated and maintained on 75% nitrous oxide ( $N_2O$ ) mixture. The flow rate was 2L/min and the circuit was open so there was no rebreathing. The brachial artery was then cannulated and the catheter tip was placed into the aortic arch for monitoring of the blood pressure. Another catheter was introduced into the femoral artery for bleeding the animals. The body temperature of the primate was monitored and maintained between 37° and 38°C by keeping the animal on a heating board.

After heparinization, the animal was bled into a transfer bag. The height of the transfer bag was adjusted to decrease or increase the rate of bleeding. The animal was bled slowly through the femoral artery so that the blood pressure dropped to 45mmHg within 20 minutes. The animal was then maintained at that level of hypotension for a total of 1 hour by infusion or withdrawing blood from the reservoir. This was then followed by another hour of hypotension during which the primate was treated with Naloxone or some other agent in a small volume. The blood pressure dropped to approximately 35mmHg at the end of the second hour of hypotension. The shed blood was then returned through a syringe (fitted with a filter) slowly over 20 minutes. The bleedout volume was approximately 22% and the blood returned was approximately 10% during the first hour. According to Dr. Reynolds, this model produces 80% mortality if the animals are treated with saline in the second hour of hypotension prior to reinfusion of shed blood.

Following our return from Iowa, we conducted additional hemorrhagic shock experiments in *Cynomolgus* monkeys using the exact procedure as outlined above (which was being carried out in Dr. Reynold's laboratory). The difference between our experiments and their experiments, however, was that we did not intubate the animal or maintain them on  $N_2O$  mixture during the hemorrhagic hypotension period. Our animals were anesthetized with ketamine and they did not require any other anesthesia except some supplemental doses of ketamine. In the second hour of hypotension we did not infuse or bleed the animals whereas in Dr. Reynold's laboratory the animals were treated with Naloxone or some other agent. Despite the fact that no treatment was given in the second hour of hypotension, none of the animals that we have studied died as a result of

hemorrhage and hypotension. As mentioned above, the only difference between our experiments and their set up was that they maintained their animals prior to and during hypovolemia on  $N_2O$  whereas we did not. It is quite possible that maintaining the animals on a nitrous oxide mixture for approximately 2 hours creates an additional stress for the animal for the following reason: The systemic effects of  $N_2O$  comprise an increase in peripheral resistance and a reduction in cardiac output with an associated reduction in total body  $O_2$  availability (Thornburn, Smith and Brown, Fr. J. Anaesth. 51:937-942, 1979). The studies of Thomson et al (Anaesthesia 37:548-553, 1982) have in fact shown that administration of  $N_2O$  caused a significant decrease in hepatic arterial, portal venous and total hepatic blood flow. Furthermore, it has been shown that  $N_2O$  produces some disoxygenation of hemoglobin (Brain Res. 213:405-444, 1981). In view of the above information, it could be concluded that  $N_2O$  itself creates a significant stress for the animals. Thus, the animals studied by Drs. Gurll and Reynolds may not have died due to hemorrhage but due to the added stress imposed by  $N_2O$ . If this is the case, it appears then that there is an artifact in the studies of Drs. Gurll and Reynolds. In view of this information, we have abandoned using the model of Drs. Gurll and Reynolds of hemorrhagic shock in the Cynomolgus monkey.

#### 15. Effect of ATP-MgCl<sub>2</sub> Infusion on Behavior and EEG in Primates.

Four experiments were performed to investigate the effect of ATP-MgCl<sub>2</sub> infusion on behavior and EEG in primates. ATP-MgCl<sub>2</sub> was administered intravenously at varying dosages and the following report summarizes the effects of this drug on the monkeys' neurological condition and his electroencephalogram. The animals, after arousing from ketamine anesthesia and being placed in a restraining chair, were allowed to become alert. Their neurological examination showed no asymmetry of movement of the forelimb and hind limbs. Grasp in each of the limbs was strong and symmetrical. There was fine lateral horizontal nystagmus, probably secondary to ketamine. Pupils were equal and round and reactive to light. The animals maintained themselves in correct posture. There was no facial asymmetry. In the first experiment monitoring took place with a needle electrode placed in the temporal bipolar montage and the second through fourth experiments were performed with a parasagittal montage with two needle electrodes placed at positions CZ and occipitally approximately one centimeter from the midline and symmetrically placed. They were not subjected to trauma during the experiments. Baseline EEG patterns were symmetrical showing good Beta activity and no sharp activity or slowing. There were occasional artifactual waves and a considerable amount of muscle artifacts when the monkeys moved their heads; however, when quiet, the recording was very adequate for monitoring the brain's electrical activity. Infusion of 0.1mg/kg/min ATP-MgCl<sub>2</sub> for seven minutes showed no cardiovascular effects and the electrical activity remained unchanged. The infusion was stopped and again no change in the EEG was noted. With the infusion of 0.5mg/kg/min ATP-MgCl<sub>2</sub>, the voltage began to decrease and slow waves appeared symmetrically as a mean arterial blood pressure began to fall to a level of 45mmHg. At that mean arterial blood pressure, there was significant depression in voltage and slow wave static throughout the period of time that the mean arterial blood pressure remained at approximately 40-50mmHg. When the infusion was stopped, the EEG pattern remained slowed and with low voltage, while the mean arterial blood pressure continued to slowly rise to 65mmHg. When the mean

arterial blood pressure reached 93mmHg, the activity picked up. There was still some low voltage activity noted. The slowing, however, had disappeared. There was gradual recovery of the EEG pattern until it returned to normal, approximately 15 minutes later, with a mean arterial blood pressure of 93mmHg. ATP-MgCl<sub>2</sub> at a rate of 1.0mg/kg/min was then infused. Again, cardiovascular effects were noted. The mean arterial blood pressure dropped to 35mmHg and EEG again slowed and the voltage decreased bilaterally. At a mean arterial blood pressure of around 30mmHg, there was a maximal depression of voltage and maximal slowing bilaterally throughout the period of time the mean arterial blood pressure was around 30mmHg. There was significant and marked depression of voltage with the occasional slow activity. Infusion was then stopped and again, as mean arterial blood pressure began to rise, there was a slow concomitant reversal of the EEG to a normal baseline appearance. However, the EEG did not return to normal until the mean arterial blood pressure was noted to be 90mmHg. After things had returned back to normal in both cardiovascular and EEG responses, 2.5mg/kg/min ATP-MgCl<sub>2</sub> was infused. There was a rapid diminishing of the EEG pattern again, and at this time, the mean arterial blood pressure dropped to 25mmHg. There was maximal depression of voltage and slowing again bilaterally and this was the most severe depression of electrical activity seen throughout the experiment. The infusion was then stopped and the arterial blood pressure began to rise along with an increase in the electrical activity. There was suppression through mean arterial pressure of 45, 55, 65 and 70mmHg following which the EEG gradually returned to normal. Again 1.0mg/kg/min ATP-MgCl<sub>2</sub> was infused and at this time the same EEG suppression following depression of blood pressure was noted. This again returned to normal as the mean arterial blood pressure rose to 90mmHg.

At the end of the experiment, the animals were alert and the EEG was back to baseline normal Beta activity. The behavioral changes noted during the experiment were consistently that of suppression of level of consciousness and drowsiness when the mean arterial blood pressure fell within the range of 20-55mmHg. The animal remained symmetrical when stimulated with a normal grasp and pupils remained reactive. The level of responsiveness increased with the return of blood pressure to a mean of 90mmHg and this was concomitant with the return to a normal EEG baseline. All four animals showed the same EEG and behavioral patterns.

#### 16. Effect of Administration of Massive and Repeated Doses of ATP-MgCl<sub>2</sub> on Survival in Rats.

We conducted additional studies in conscious rats in which a bolus amount of the entire efficacious dose of ATP-MgCl<sub>2</sub> was injected rapidly intravenously (50umoles/kg/ BW). Although the blood pressure of such animals dropped to approximately 40mmHg transiently, none of the animals studied died as a result of ATP-MgCl<sub>2</sub> infusion.

In additional studies, we injected the rats daily with the bolus amount of the entire efficacious dose of ATP-MgCl<sub>2</sub> for a total of 5 days. The jugular vein, in these experiments, was cannulated and the catheter was tunneled through the skin and the muscle and made to come out through the rat's back. The sealed catheter tip was cut daily and the catheter connected to a syringe containing ATP-MgCl<sub>2</sub> prior to infusion. Thus, with this procedure the animals received

ATP-MgCl<sub>2</sub> daily without the need of anesthesia. The results showed that there was no apparent long-term side effect of ATP-MgCl<sub>2</sub> infusion in these animals in comparison to rats receiving saline infusion daily for the same period of time.

In other studies, we infused a total of eight times the efficacious dose of ATP-MgCl<sub>2</sub> over a very short period of time in conscious rats. Despite this massive dose, none of the animals died or showed any long-term adverse side effects. Although the blood pressure of such rats dropped with the massive infusions of ATP-MgCl<sub>2</sub>, it returned to normal shortly thereafter. Thus, there does not appear to be any long-term adverse effects of ATP-MgCl<sub>2</sub> on survival or behavior of animals. In addition, bolus amounts of ATP-MgCl<sub>2</sub> as well as repeated infusions of ATP-MgCl<sub>2</sub> did not produce mortality in the otherwise normal animals.

17. Effect of Daily Administration of ATP-MgCl<sub>2</sub> for Prolonged Periods of Time.

We have also conducted additional studies in conscious rats in which the entire efficacious dose of ATP-MgCl<sub>2</sub> (50μmoles/kg BW) was injected rapidly intraperitoneally once a day (5 times a week) for a period of 3 months. Since keeping the indwelling catheters patent for a period of 3 months would have been a problem, we had to resort to injection the ATP-MgCl<sub>2</sub> solution intraperitoneally. Such rats demonstrated no observable ill effects of daily ATP-MgCl<sub>2</sub> infusion. They ate, drank and gained weight in the same manner as normal rats given daily intraperitoneal saline injections. Thus, there does not appear to be any harmful effects of even daily administration of ATP-MgCl<sub>2</sub> for prolonged periods of time.

18. Effect of Bolus Administration of ATP-MgCl<sub>2</sub> on survival in Primates.

We have also conducted studies in primates in which the entire efficacious dose of ATP-MgCl<sub>2</sub> (50μmoles/kg BW) was injected rapidly intravenously (50μmoles/kg BW). Under ketamine anesthesia, a femoral artery and vein were cannulated for measurement of arterial blood pressure and infusion of ATP-MgCl<sub>2</sub>, respectively. Mean arterial pressure decreased to approximately 35mmHg during the rapid infusion of ATP-MgCl<sub>2</sub>. Upon completion of infusion mean arterial pressure returned to preinfusion levels within 1 to 3 minutes. There were no mortalities due to bolus infusion of ATP-MgCl<sub>2</sub> either immediately following the infusion or for at least two weeks thereafter at which point the observations were terminated. Thus, both species of animals, i.e. rats and primates, tolerated the bolus and repeated infusions of ATP-MgCl<sub>2</sub> and there were no apparent side effects.

19. FDA Approval of ATP-MgCl<sub>2</sub> for Phase I Studies.

We submitted an application to the FDA seeking permission to use ATP-MgCl<sub>2</sub> in normal human volunteers. The FDA informed us on May 2, 1983 that our application for using ATP-MgCl<sub>2</sub> in clinical studies was approved. Attached is a copy of the letter of approval from the FDA.

## 20. Human Investigation Committee Approval.

We also submitted the protocol to our Human Investigation Committee for their approval of ATP-MgCl<sub>2</sub> for Phase I clinical studies. The application was reviewed by the full committee and approved on March 29, 1983. We also submitted the protocol of our study to the Army's Human Investigation Committee and the protocol was approved.

## 21. Phase I Studies of ATP-MgCl<sub>2</sub>.

Since the approval of the Phase I studies by our Human Investigation Committee and the U.S. Army Medical Research and Development Command, we completed the four components of the Phase I studies. All five volunteers who had signed the consent form and participated in that study received ATP-MgCl<sub>2</sub> on four separate occasions with a total dose of 3, 6, 10 and 30mg/kg (20 studies).

The volunteers underwent a physical examination by a primary care physician prior to receiving ATP-MgCl<sub>2</sub> and they were all found to be free of any renal or cardiovascular problems. All Phase I studies were carried out in the operating room at Yale-New Haven Hospital solely for the benefit of having the facilities available in the unlikely event that ventilatory and cardiac support would be required during the study.

On the day of each study, two intravenous catheters were placed, under sterile conditions, in the forearm veins and ATP-MgCl<sub>2</sub> was infused through one of the catheters. Blood sampling and injection of dye (indocyanine green) for cardiac output determinations were carried out through the second catheter. Each volunteer had his baseline values of sodium, potassium, glucose, hemoglobin, blood pressure, heart rate and cardiac output recorded just prior to receiving ATP-MgCl<sub>2</sub>. ATP-MgCl<sub>2</sub> was infused intravenously at rates of 0.1, 0.2, 0.25, 0.28, 0.32, 0.38, 0.40 and 0.56mg/kg/min. Vital signs were recorded every 3 minutes except at the high-dose ATP-MgCl<sub>2</sub> infusion during which they were obtained every minute. Each infusion rate was carried out usually for ten minutes. In the final study (full dose of ATP-MgCl<sub>2</sub>), however, each infusion rate was carried out for 20 or 30 minutes. Infusions were stopped for 5 minutes prior to switching to higher infusion rates. Blood samples were taken during each ATP-MgCl<sub>2</sub> infusion and cardiac output was measured using the Nihon-Kohden cardiac output computer at various ATP-MgCl<sub>2</sub> infusion rates. Five minutes after the last ATP-MgCl<sub>2</sub> infusion, blood samples were obtained and cardiac output determined in addition to recording vital signs. In addition, blood samples were obtained from volunteers one week after the administration of the full dose of ATP-MgCl<sub>2</sub> and SGOT and SGPT levels were determined. Statistical analysis was performed with ANOVA and coefficients of correlation. Statistical significance was attributed to values of  $p < 0.05$ .

## General Observations

With the infusion of 0.1mg/kg/min ATP-MgCl<sub>2</sub> and higher, most subjects experienced a feeling of slight chest congestion, flushing in the face, overall warmth and light-headedness. The intensity of the symptoms, however, decreased with the continuation of ATP-MgCl<sub>2</sub> infusion and all of the above

symptoms disappeared within a minute or two after the ATP-MgCl<sub>2</sub> infusion was stopped or completed. The mean arterial blood pressure, electrolytes, hemoglobin and serum glucose levels did not change significantly even with the continuous infusion of ATP-MgCl<sub>2</sub>. The heart rate and cardiac output, however, increased with the increase in ATP-MgCl<sub>2</sub> infusion rates.

In the final study (i.e. the full dose of ATP-MgCl<sub>2</sub>), most subjects experienced a feeling of chest congestion, flushing of the face, and light-headedness. The intensity of these symptoms, however, decreased during the ATP-MgCl<sub>2</sub> infusion. Most subjects also had a feeling of transient nausea if the ATP-MgCl<sub>2</sub> infusion rates were above 0.30mg/kg/min. In one subject, when the ATP-MgCl<sub>2</sub> infusion rates were increased to 0.56mg/kg/min, the subject vomited during the infusion. The rate of ATP-MgCl<sub>2</sub> infusion in the subsequent volunteers was therefore kept below 0.5mg/kg/min and none of the other volunteers had any vomiting as a result of ATP-MgCl<sub>2</sub> infusion. In addition to the above mentioned symptoms, most subjects experienced increased intestinal motility. This was probably due to the effects of ATP-MgCl<sub>2</sub> on the intestinal smooth muscle. Nonetheless, despite the small uncomfortableness, all five subjects tolerated the ATP-MgCl<sub>2</sub> infusion and the slight discomfort they experienced during the high dose ATP-MgCl<sub>2</sub> infusions disappeared shortly after completion of the infusion. Our studies have thus indicated that ATP-MgCl<sub>2</sub> infusion is well tolerated by normal subjects, provided that the rate of infusion is not above 0.5mg/kg/min. No ventilatory or cardiac support was required in any subject during any of the ATP-MgCl<sub>2</sub> infusion studies.

Measurement of serum GOT and GPT levels before, during, at the end of ATP-MgCl<sub>2</sub> infusion and one week after the last ATP-MgCl<sub>2</sub> infusion indicated that the level of these enzymes were not affected by the administration of ATP-MgCl<sub>2</sub>. In addition, there was no adverse effect of ATP-MgCl<sub>2</sub> on renal function and there were no delayed side effects of ATP-MgCl<sub>2</sub> infusion.

#### Effects of ATP-MgCl<sub>2</sub> infusion on heart rate.

The percent increase in heart rate (HRPCT) versus ATP-MgCl<sub>2</sub> infusion rates (INFUSRT) is presented in Figure 1. The numbers 1,2,3,4, and 5 represent the code for each volunteer. The results indicated that the heart rate increased with the increase in ATP-MgCl<sub>2</sub> infusion rates. There was a good correlation between ATP-MgCl<sub>2</sub> infusion rates and the increase in heart rate with an r value of 0.72 (p < 0.001).

Plotting the percent increase in heart rate (HRPCT) versus percent change in cardiac output (COPCT) indicated that the correlation between these two parameters was not very good (Fig. 2). The r value was found to be 0.55.

From these results, it is clear that maximal increase in heart rate was observed when ATP-MgCl<sub>2</sub> was infused at a rate of approximately 0.3mg/kg/min and with the infusion of 0.1mg/kg/min ATP-MgCl<sub>2</sub>, the increase in heart rate was marginal.

The absolute heart rate versus ATP-MgCl<sub>2</sub> infusion rate is presented in Fig. 3. It is clear from this figure that only in two subjects the heart rate exceeded 130 with the infusion of 0.38mg/kg/min ATP-MgCl<sub>2</sub>. With the infusion



of 0.1mg/kg/min ATP-MgCl<sub>2</sub>, the heart rate in any of the subjects did not increase over 100 beats/min. There was a poor correlation between the absolute heart rate and the total dose of ATP-MgCl<sub>2</sub> infused (Fig. 4). The r value in this case was found to be 0.22.

#### Effects of ATP-MgCl<sub>2</sub> infusion on mean blood pressure.

In Figure 5 the percent change in mean blood pressure versus ATP-MgCl<sub>2</sub> infusion rates is plotted. The results indicated that there was no correlation between ATP-MgCl<sub>2</sub> infusion rates and the mean blood pressure. The r value was found to be -0.05.

There was also no correlation between the percent change in mean blood pressure and the total amount of ATP-MgCl<sub>2</sub> infused (Fig. 6). The r value was found to be 0.03.

Plotting the percent change in mean blood pressure versus percent change in cardiac output (COPCT) indicated that there was no correlation between the changes in mean blood pressure and cardiac output. The r value was found to be -0.09 (Fig. 7).

From these results, it is clear that the mean blood pressure did not change significantly with the infusion of ATP-MgCl<sub>2</sub>.

Since the heart rate increased with ATP-MgCl<sub>2</sub> infusion, it could be suggested that the observed increase in cardiac output may be due to the increase in heart rate. Since there was no significant change in mean blood pressure with ATP-MgCl<sub>2</sub> infusion but cardiac output increased significantly, it indicates that ATP-MgCl<sub>2</sub> must be producing peripheral vasodilatation and the increase in the heart rate may be due to sympathetic system stimulation.

#### Effect on Systemic Vascular Resistance (SVR).

SVR decreased significantly with the increase in ATP-MgCl<sub>2</sub> infusion rates. The plot of SVR versus cardiac output (CO) indicates that there is a good correlation between those parameters (Fig. 8). The r value was found to be 0.89. The decreased SVR during ATP-MgCl<sub>2</sub> infusion may also be contributing to the increase of CO. Thus, the increased CO appears to be compensating for the decrease of SVR and hence maintaining the mean blood pressure during ATP-MgCl<sub>2</sub> infusion.

#### Effect of ATP-MgCl<sub>2</sub> on Stroke Volume Index and Other Parameters.

The results presented in Table I indicate that cardiac output increased by 77% (p 0.0001) at maximum infusion rate. This was paralleled by an increase in heart rate (45%, p 0.0001). Stroke volume index increased by 14% (p 0.05). However, the mean blood pressure did not change significantly over the entire range of infusion rates. Systemic vascular resistance decreased 56% at the highest rate of ATP-MgCl<sub>2</sub> infusion. A mean infusion rate of 0.32±0.02mg/kg/min was associated with maximum increases in heart rate (52%) and cardiac output (119%) without affecting mean blood pressure.

The results of this study demonstrate that the increase in cardiac output offset the decrease in systemic vascular resistance. Thus, blood pressure (MBP) was maintained. Furthermore, the increase in stroke volume index demonstrated a mild inotropic effect. Thus, the pharmacologic profile of vasodilatation, augmentation of cardiac output, maintenance of blood pressure and mild positive inotropy coupled with beneficial metabolic effects in animals suggests an important therapeutic role of ATP-MgCl<sub>2</sub> in the treatment of conditions characterized by regional or global ischemia.

#### Effects of ATP-MgCl<sub>2</sub> infusion on cardiac output.

The results presented in Figure 9 are expressed as percent increase in cardiac output versus ATP-MgCl<sub>2</sub> infusion rates in mg/kg/min. As can be seen from this figure, cardiac output increased progressively with the increase in ATP-MgCl<sub>2</sub> infusion rates and there was a good correlation between the increase in cardiac output and ATP-MgCl<sub>2</sub> infusion rates. The  $r$  value was found to be 0.75 ( $p < 0.001$ ). The correlation between percent increase in cardiac output and the total dose of ATP-MgCl<sub>2</sub> infused was not good ( $r=0.20$ ) (Fig. 10). Thus, the increase in cardiac output was dependent upon the rate of ATP-MgCl<sub>2</sub> infusion but not on the total dose of ATP-MgCl<sub>2</sub> infused. From the results presented in Fig. 9, it appears that maximal increase in cardiac output in normal volunteers was obtained with the infusion of approximately 0.3mg/kg/min ATP-MgCl<sub>2</sub> and that approximately 10% increase in cardiac output was observed when ATP-MgCl<sub>2</sub> was infused at a rate of 0.1mg/kg/min.

#### Effects of ATP-MgCl<sub>2</sub> on sodium, potassium, blood glucose and hemoglobin concentration.

The percent changes in sodium (NAPPCT) versus ATP-MgCl<sub>2</sub> infusion rates (INFUSRT) is plotted in Fig. 11 and, as can be seen, there was no correlation between the percent change in sodium and ATP-MgCl<sub>2</sub> infusion rates. The  $r$  value in this case was found to be 0.04. There was also no correlation between NAPPCT and total dose of ATP-MgCl<sub>2</sub> infused (Fig. 12). The  $r$  value in this case was -0.12. Likewise, there was no correlation between the percent change in potassium (KPPCT) and ATP-MgCl<sub>2</sub> infusion rates (Fig. 13) and total dose (Fig. 14). The  $r$  values in these cases were found to be 0.02 and -0.12, respectively.

The percent change in glucose (GLUCPCT) versus ATP-MgCl<sub>2</sub> infusion rates is plotted in Fig. 15 and, as can be seen, there was no correlation between these two parameters. The  $r$  value in this case was found to be 0.28. Similarly, there was no correlation between GLUCPCT and total dose of ATP-MgCl<sub>2</sub> infused ( $r=0.16$ ) (Fig. 16).

The percent change in hemoglobin (HBPCT) versus ATP-MgCl<sub>2</sub> infusion rate (INFUSRT) is plotted in Fig. 17 and, as can be seen, there was no correlation between these two parameters. The  $r$  value in this case was found to be 0.35. Likewise, the correlation between HBPCT and total dose of ATP-MgCl<sub>2</sub> infused was not good ( $r=0.26$ ) (Fig. 18). These results therefore indicate that there were no significant changes in blood levels, sodium, potassium or hemoglobin contents with ATP-MgCl<sub>2</sub> infusion.

### Effect of ATP-MgCl<sub>2</sub> infusion on serum GOT and GPT levels.

Measurement of serum GOT and GPT during different rates of ATP-MgCl<sub>2</sub> infusion and at 7 days after ATP-MgCl<sub>2</sub> administration revealed that there were no changes in the levels of the above enzymes during or after ATP-MgCl<sub>2</sub> administration (Table II).

### Summary of the Phase I studies.

Our studies have indicated ~~that~~, depending on the dose of ATP-MgCl<sub>2</sub> infusion, most subjects experienced a feeling of slight chest congestion, increased intestinal motility, flushing of the face, light-headedness and occasionally a feeling of transient nausea. The intensity of these symptoms, however, decreased with the continuation of the same dose of ATP-MgCl<sub>2</sub> infusion. All the above symptoms disappeared within a minute or two after the ATP-MgCl<sub>2</sub> was discontinued or completed. There was no significant change in mean arterial blood pressure, sodium, potassium, hemoglobin and blood glucose levels. However, the heart rate and the cardiac output increased progressively with the increase in ATP-MgCl<sub>2</sub> infusion rates. Our studies have also indicated that infusion of greater than 0.5mg/kg/min ATP-MgCl<sub>2</sub> may cause vomiting and severe discomfort and, thus, ATP-MgCl<sub>2</sub> infusions in man should be carried out below the rate of 0.5mg/kg/min. None of the volunteers required any ventilatory or cardiac support during any of the studies and all volunteers tolerated the ATP-MgCl<sub>2</sub> infusion. There were no delayed side effects of ATP-MgCl<sub>2</sub> infusion in any of the volunteers. In addition, none of the subjects requested that the study be terminated. These results have therefore demonstrated that it is safe to administer ATP-MgCl<sub>2</sub> in normal volunteers.

The results also suggest that while it is possible to administer ATP-MgCl<sub>2</sub> in normal volunteers at a rate of up to 0.4mg/kg/min without any significant adverse effects, such higher rates of ATP-MgCl<sub>2</sub> administration may not be advisable in certain subsets of patients. In patients in whom large increases in heart rate may have adverse hemodynamic effects, the rate of ATP-MgCl<sub>2</sub> infusion should be 0.1-0.2mg/kg/min. Thus, infusion of ATP-MgCl<sub>2</sub> in such patients should be carried out with monitoring of the heart rate.

In conclusion, data from this study suggest a potentially beneficial role for ATP-MgCl<sub>2</sub> in the treatment of low flow states and confirm the safety of ATP-MgCl<sub>2</sub> in humans.

### 22. Approval of Phase II Studies of ATP-MgCl<sub>2</sub> by our Human Investigation Committee.

We submitted the protocol to our Human Investigation Committee for their approval of ATP-MgCl<sub>2</sub> for Phase II studies. The application was reviewed by the full committee and approved on June 14, 1984.

23. Submission of our protocol for Phase II studies of ATP-MgCl<sub>2</sub> to the Army's Human Investigation Committee.

We also submitted our protocol for Phase II studies of ATP-MgCl<sub>2</sub> to the U.S. Army's Human Investigation Committee for their approval. The application was reviewed and approved.

24. Food and Drug Administration.

We also requested the FDA to permit us to use yeast ATP instead of muscle ATP in our Phase II studies of ATP-MgCl<sub>2</sub>. The reason for this is as follows:

In most of our previous experimental studies with ATP-MgCl<sub>2</sub> we have used the disodium ATP obtained from equine muscle. This source of ATP was used since it was considered to be the highest purity ATP available at the time we initiated our studies. Recently, Sigma Chemical Co. has been able to obtain ATP from yeast which is also 99-100% pure. Since ATP obtained from yeast is far less expensive than ATP from equine muscle, we conducted additional studies in which we compared the effects of yeast versus muscle ATP on hepatic mitochondrial function and blood flow following hepatic ischemia. The results indicated that the improvement in mitochondrial function as well as in hepatic blood flow following ischemia and treatment with yeast or muscle ATP was the same. Thus, it is clear that the beneficial effects of ATP-MgCl<sub>2</sub> following adverse circulatory conditions are not dependent on the source of ATP. The efficacy of yeast ATP is of potentially far-reaching practical importance since we are in the process of initiating a large number of clinical studies with ATP-MgCl<sub>2</sub>. Although the isolation of ATP from equine muscle has been adequate to meet the needs imposed by research demands, there may be limitations for large-scale production for clinical use. Yeast ATP, on the other hand, can be produced in large quantities by phosphorylation of adenosine by yeast and has the added advantage of being far less expensive than ATP isolated from equine muscle. Thus, the use of this source of ATP would make potential ATP-MgCl<sub>2</sub> treatment far more cost-effective. Our request was approved.

25. Effect of ATP-MgCl<sub>2</sub> on myocardial O<sub>2</sub> consumption and coronary blood flow.

In the Phase I studies of ATP-MgCl<sub>2</sub> infusion in normal volunteers, most volunteers demonstrated tachycardia during the infusion of this agent. It could thus be argued that tachycardia which was observed with ATP-MgCl<sub>2</sub> infusion and its attendant effects on myocardial oxygen demand may well set the stage for exacerbation of any deleterious mismatch between myocardial oxygen demand and myocardial oxygen supply. Thus, the potential results could be dysrhythmias, myocardial ischemia, myocardial infarction or all three. Since the Phase I studies of ATP-MgCl<sub>2</sub> did not eliminate this possibility, we found it necessary to conduct additional animal experiments to determine whether or not ATP-MgCl<sub>2</sub> increases myocardial O<sub>2</sub> consumption. To study this, 9 closed-chest, mongrel dogs were anesthetized with Nembutal and instrumented with a BAIM catheter in the coronary sinus for measurement of coronary flow (CF) and sampling of coronary sinus blood and a Swan Ganz catheter for cardiac output (CO) measurement. Arterial blood pressure was monitored via catheter in the femoral

artery. ATP-MgCl<sub>2</sub> (mg/kg/min) or Nitroprusside (NP) (ug/kg/min) were infused into a femoral vein. In additional experiments, dogs were made hypovolemic (mean arterial pressure (MAP) of 80 ± 5 mmHg) and the ATP-MgCl<sub>2</sub> infusion repeated. All results are normalized to percent of the immediately preceding baseline values. The results of coronary flow, myocardial oxygen consumption (MVO<sub>2</sub>), mean arterial pressure, cardiac output and an estimate of myocardial energy cost (MVO<sub>2</sub>/heart rate x peak systolic pressure) were:

Infusion Rate	CF	MVO <sub>2</sub>	MAP	CO	Energy Cost
<u>ATP-MgCl<sub>2</sub></u>					
0.6	123 ± 19	59 ± 11	81 ± 3	154 ± 16	74%
1.2	245 ± 15	53 ± 9	70 ± 3	143 ± 8	74%
2.5	182 ± 24	39 ± 3	60 ± 4	131 ± 6	67%
2.5 (Hypovolemic)	198 ± 50	63 ± 22	57 ± 1	120 ± 5	98%
<u>Nitroprusside</u>					
5	88 ± 10	80 ± 15	76 ± 6	118 ± 12	110%
10	130 ± 40	87 ± 16	65 ± 5	120 ± 13	143%
20	95 ± 9	51 ± 6	45 ± 6	85 ± 3	94%

These results demonstrate that, even during hypotension, ATP-MgCl<sub>2</sub> increases CF and CO while decreasing MVO<sub>2</sub>. Although both ATP-MgCl<sub>2</sub> and NP reduced MVO<sub>2</sub> through after-load reduction (decreased work), ATP-MgCl<sub>2</sub> but not NP decreases MVO<sub>2</sub> for any given workload. This indicates an additional metabolic effect of ATP-MgCl<sub>2</sub>. This combination of increased CO with decreased MVO<sub>2</sub> supports a role for therapeutic use of ATP-MgCl<sub>2</sub> during low-flow states and with coronary insufficiency. The results presented above therefore clearly indicate that ATP-MgCl<sub>2</sub> administration does not cause any deleterious mismatch between myocardial oxygen demand and myocardial oxygen supply.

#### 26. Effect of ATP-MgCl<sub>2</sub> infusion on total body oxygen consumption and hemodynamics.

We have also determined whether ATP-MgCl<sub>2</sub> administration has any deleterious effects on total body oxygen consumption. To study this, six adult mongrel dogs were anesthetized with sodium pentobarbital using a 20mg/kg initial bolus and a 5mg/kg/hr constant infusion. They were ventilated on a Harvard respirator set at a tidal volume of 5cc/kg and a respirator rate of 10-12/min. Hemodynamic and respiratory parameters were monitored using indwelling pulmonary artery, femoral artery and femoral vein catheters and a Beckman metabolic cart, respectively. After baseline measurements were obtained, ATP-MgCl<sub>2</sub> was infused at rates from 0.32mg/kg/min to 2.56mg/kg/min. Comparing baseline data and data obtained during the highest infusion rate, systemic vascular resistance (mean SE) (dyn-sec-cm<sup>-5</sup>) decreased from 4,720 ± 853 to 2,010 ± 1021 (P < 0.02 by the paired t-test). Mean arterial pressure (mmHg) decreased from 159 ± 4 to 97 ± 14 (P < 0.01). Heart rate decreased from 164 ± 14 to 124 ± 14 (P < 0.01). Cardiac output (L/min) by the Fick method, increased from 3.01 ± 0.42 to 3.87 ± 0.44 (P < 0.01). Oxygen pulse (ml/beat) increased from 0.79 ± 0.07 to 0.95 ± 0.09 (P < 0.01) and oxygen delivery (ml/min) increased from 590 ± 102 to 750 ± 114 (P < 0.01).

( $P < 0.01$ ). There was no significant change in pulmonary vascular resistance, pulmonary capillary wedge pressure or right atrial pressure. Oxygen consumption (ml/min) decreased from  $127 \pm 9$  to  $114 \pm 11$  ( $P < 0.005$ ) while carbon dioxide production did not change. Arterial blood gases showed no significant change in pH or  $PCO_2$  but  $PO_2$  (mmHg) increased from  $102 \pm 2$  to  $111 \pm 3$  ( $P < 0.01$ ), due to an increase in mixed venous  $PO_2$  from  $42 \pm 2$  to  $52 \pm 1$  ( $P < 0.01$ ). We conclude that ATP infusion decreases oxygen consumption despite a concomitant increase in cardiac output and oxygen delivery. This could well be a metabolic effect of infused ATP-MgCl<sub>2</sub>.

## 27. Phase II Studies of ATP-MgCl<sub>2</sub>.

We evaluated the safety of ATP-MgCl<sub>2</sub> administration in six patients with coronary artery disease. ATP-MgCl<sub>2</sub> infusion was carried out into the left main coronary artery of such patients during routine diagnostic coronary angiography procedures. Concentrations of 0.01 mg/kg/min infused over a 5 minute period were tolerated without symptomatic effects on each patient. There was no measurable effect on blood pressure, heart rate or cardiac output which all remained at baseline levels. There was no visible contractile abnormality during the ATP-MgCl<sub>2</sub> infusion as monitored by two-dimensional echocardiography.

Concentrations of 0.05 mg/kg/min in one patient (who was diabetic) produced a minor fall in blood pressure of approximately 10mmHg, bradycardia (heart rate falling from 80 beats/min to 60 beats/min) and was accompanied by the subject feeling chest congestion. The bradycardia and symptomology disappeared promptly (within seconds) upon cessation of the drug infusion (which we had been infusing for up to 2 minutes). In all the other patients the infusion rate was kept below 0.04 mg/kg/min and there was no decrease in blood pressure, bradycardia or any evidence of ischemia during ATP-MgCl<sub>2</sub> infusion.

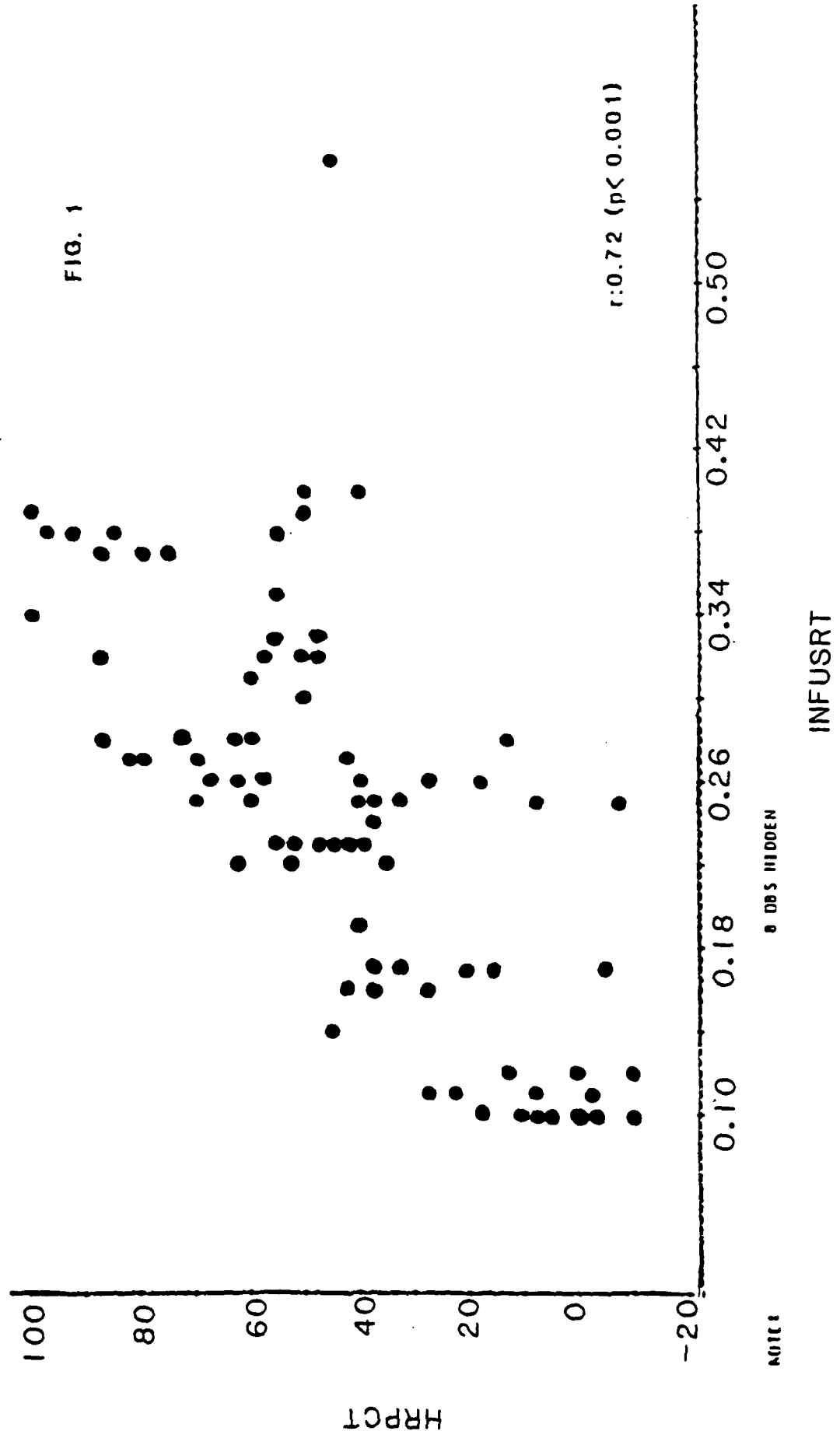
In three patients, coronary sinus catheters were inserted and the effects of ATP-MgCl<sub>2</sub> infusion on coronary sinus blood flow and myocardial O<sub>2</sub> consumption were investigated. ATP-MgCl<sub>2</sub> in such patients was infused into the left coronary artery at rates from 0.01 - 0.37 mg/kg/min. The results indicated that heart rate, blood pressure, cardiac output and pulmonary capillary blood pressure remained unchanged from pre-infusion values. The results (mean  $\pm$  SE) of coronary sinus blood flow, myocardial O<sub>2</sub> consumption and coronary vascular resistance were as follows:

	<u>CSBF</u>	<u>MVO<sub>2</sub></u>	<u>CVR</u>
Pre-ATP-MgCl <sub>2</sub> Infusion:	$178 \pm 11$ ml/min	$14.9 \pm 2.9$ cc/min	$0.6 \pm 0.01$
During ATP-MgCl <sub>2</sub> Infusion:	$293 \pm 30$ ml/min*	$10.9 \pm 2.2$ cc/min*	$0.36 \pm 0.01^*$

\*  $p < 0.05$  compared to pre-ATP-MgCl<sub>2</sub> infusion.

CSBF = Coronary sinus blood flow  
MVO<sub>2</sub> = Myocardial O<sub>2</sub> consumption  
CVR = Coronary vascular resistance

These data demonstrate a 65% increase in coronary sinus blood flow with a concomitant 27% reduction in myocardial  $O_2$  consumption, indicating that ATP-MgCl<sub>2</sub> is a demand-independent coronary vasodilator. The reduction in myocardial  $O_2$  consumption in the absence of changes in the measured determinants of myocardial oxygen demand suggest a possible oxygen-sparing effect of ATP-MgCl<sub>2</sub>. These results therefore indicate that ATP-MgCl<sub>2</sub> can be infused safely in patients with coronary artery disease and that infusion of ATP-MgCl<sub>2</sub> up to 0.037 mg/kg/min does not produce any bradycardia or decrease in blood pressure but does increase coronary sinus blood flow and decreases myocardial  $O_2$  consumption. Thus, ATP-MgCl<sub>2</sub> shows favorable characteristics for potential application in patients with coronary artery disease.





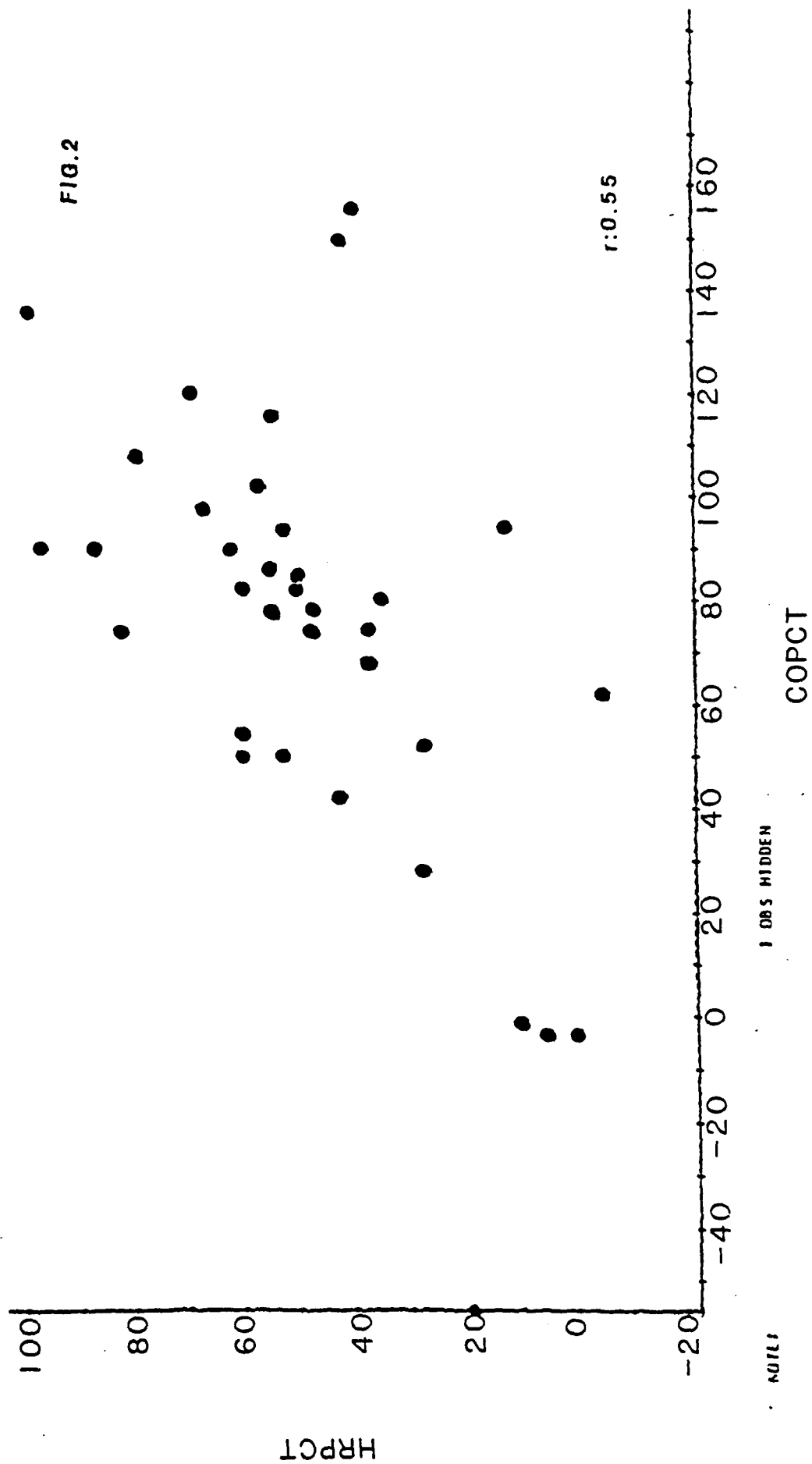


FIG. 3

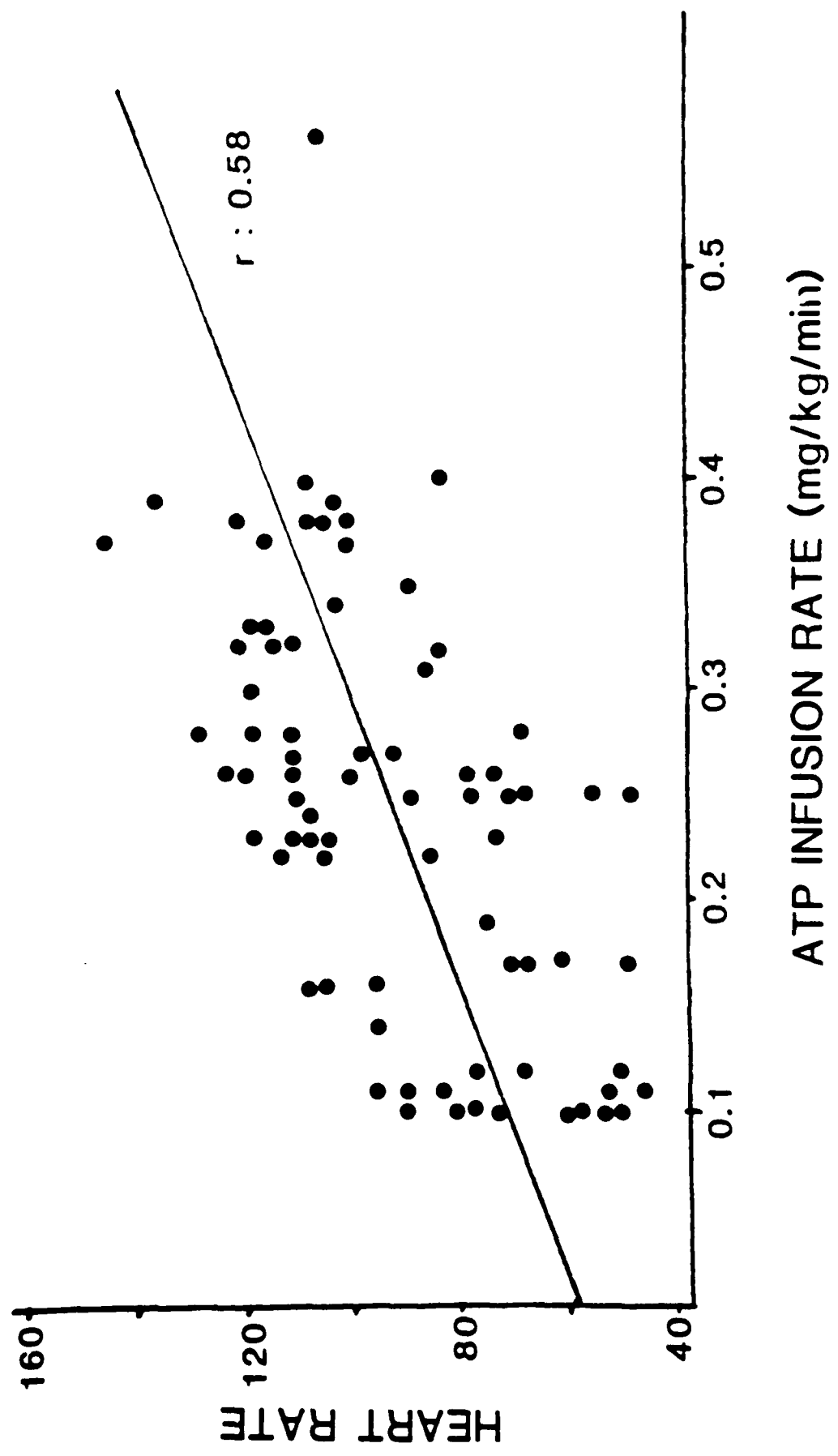


FIG. 4

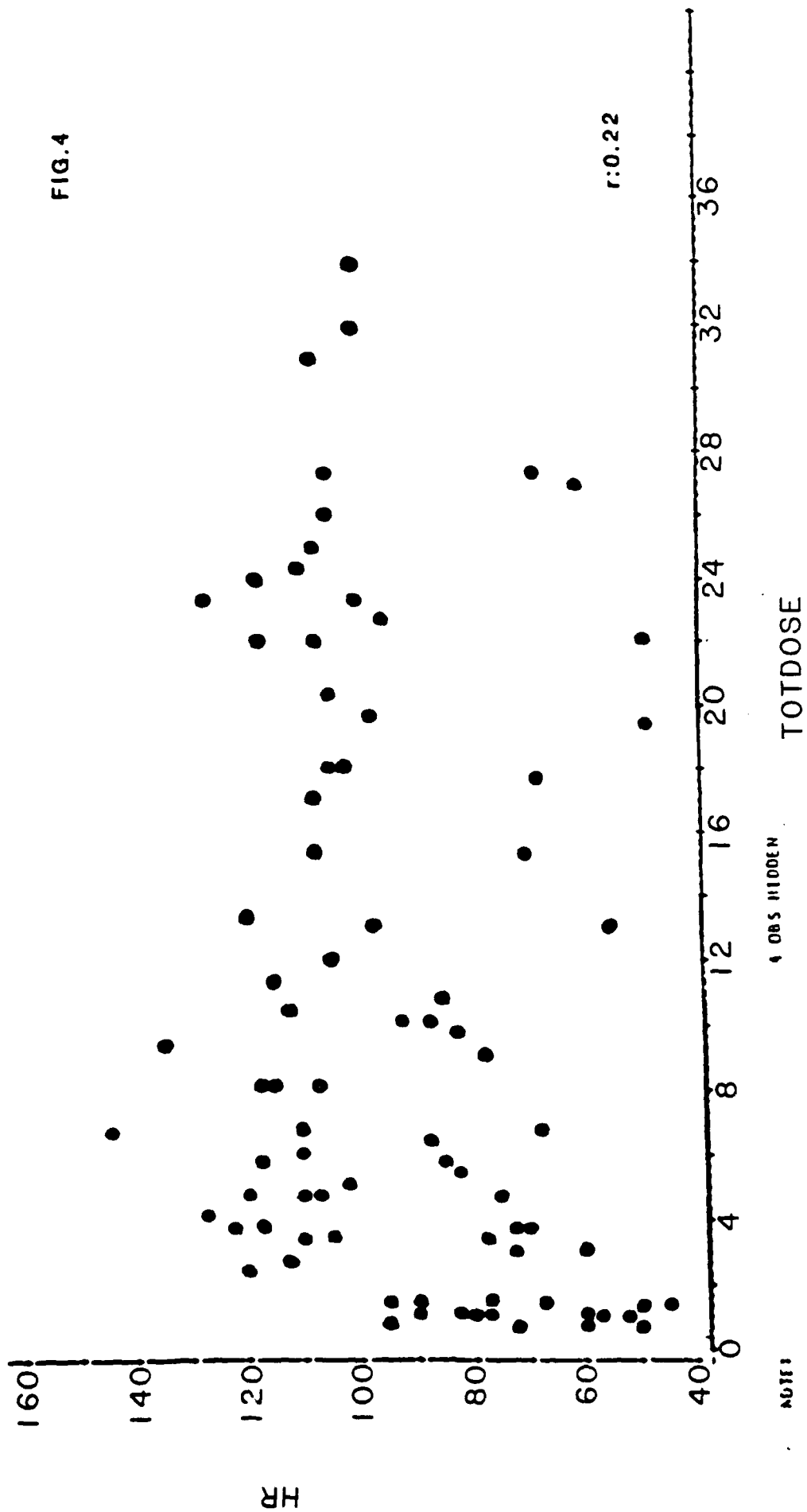
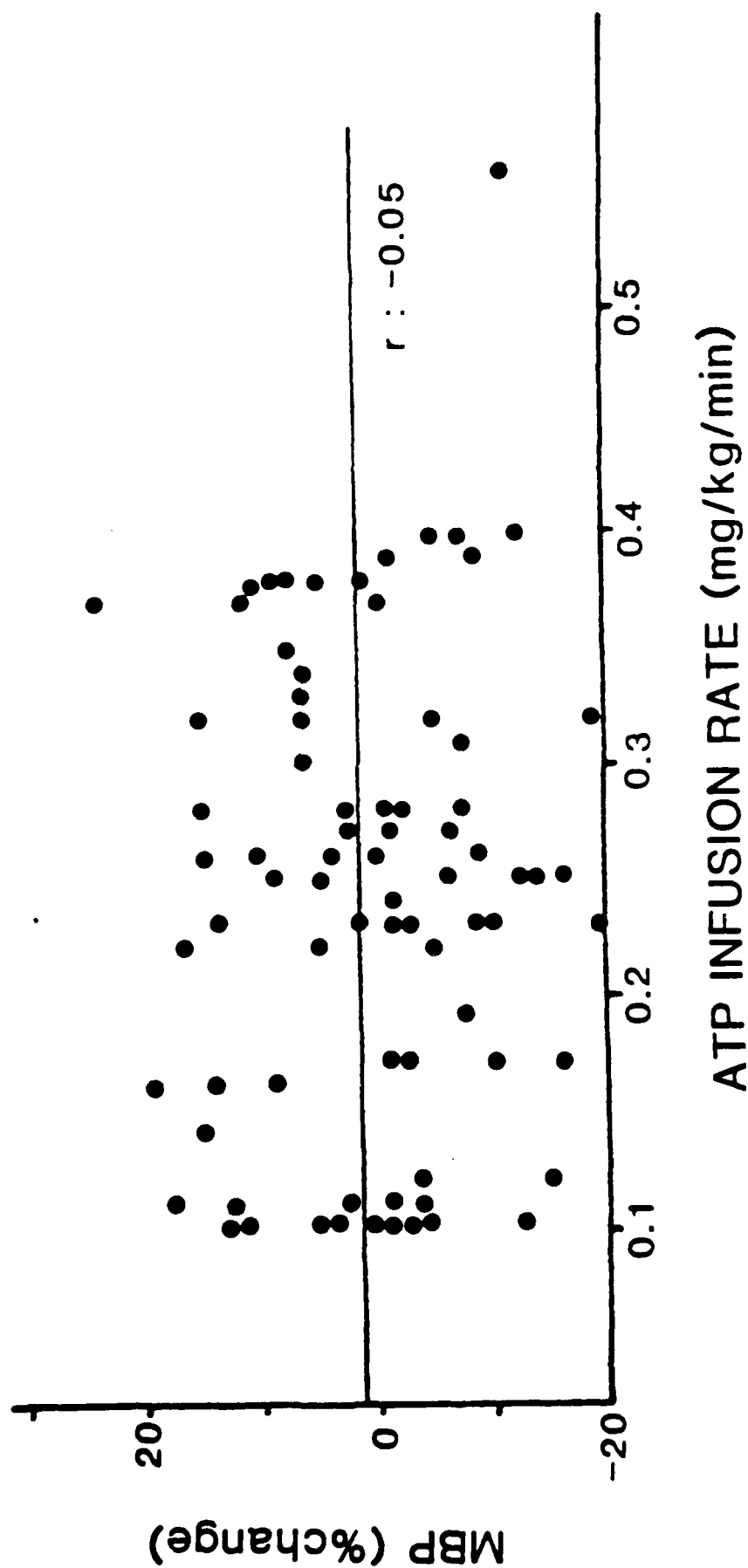
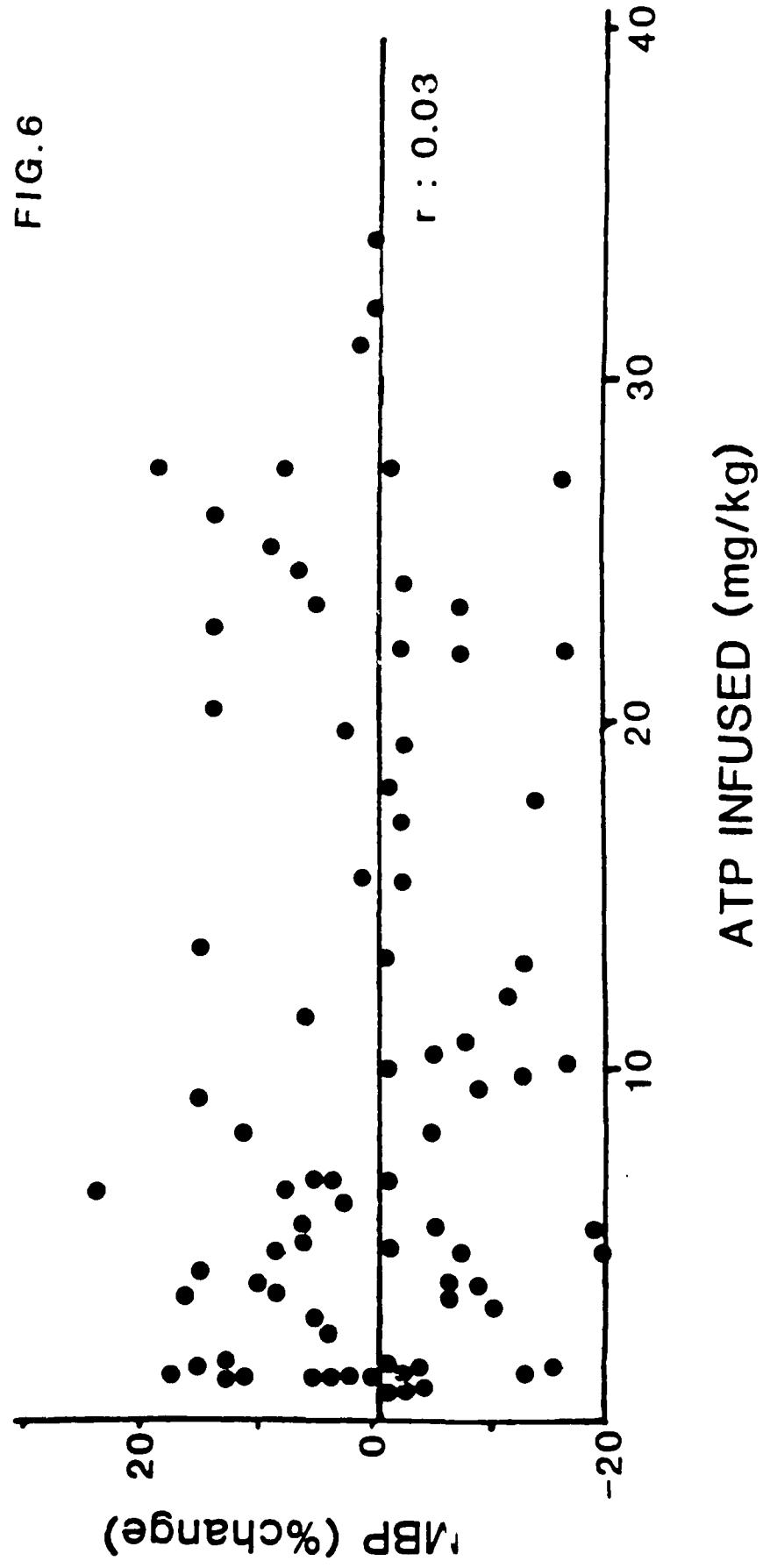
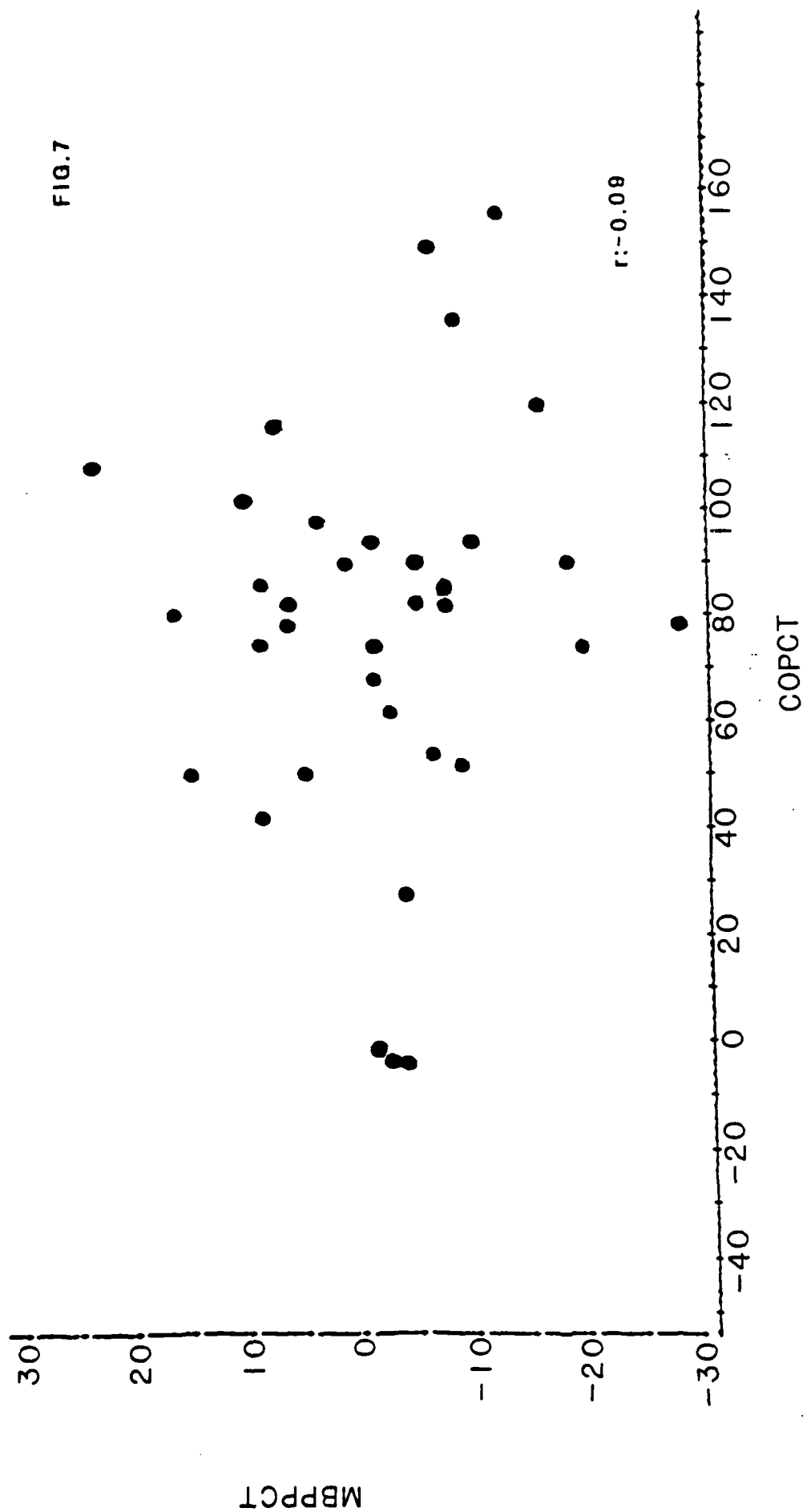
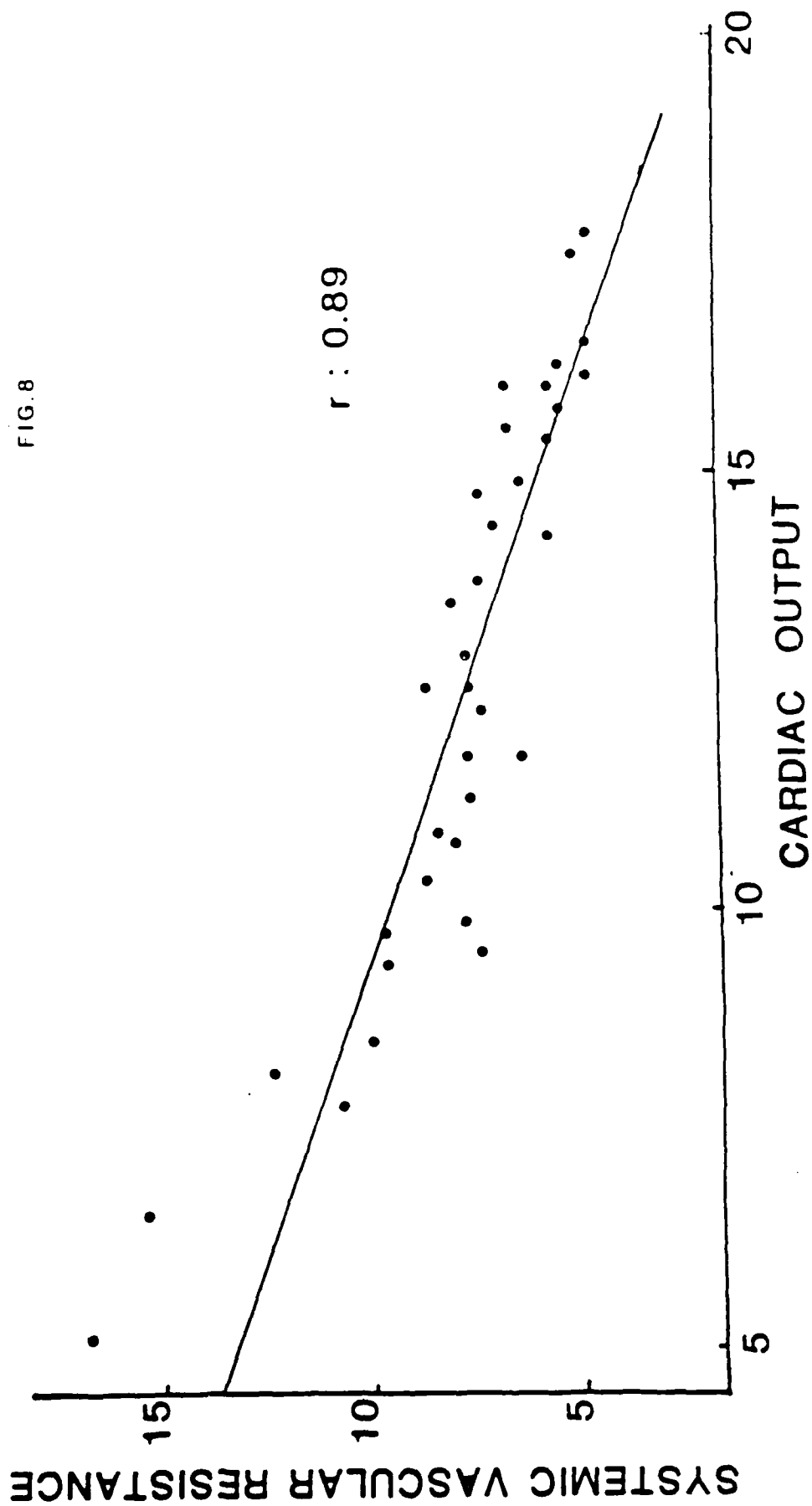


FIG. 5









**FIG. 9**

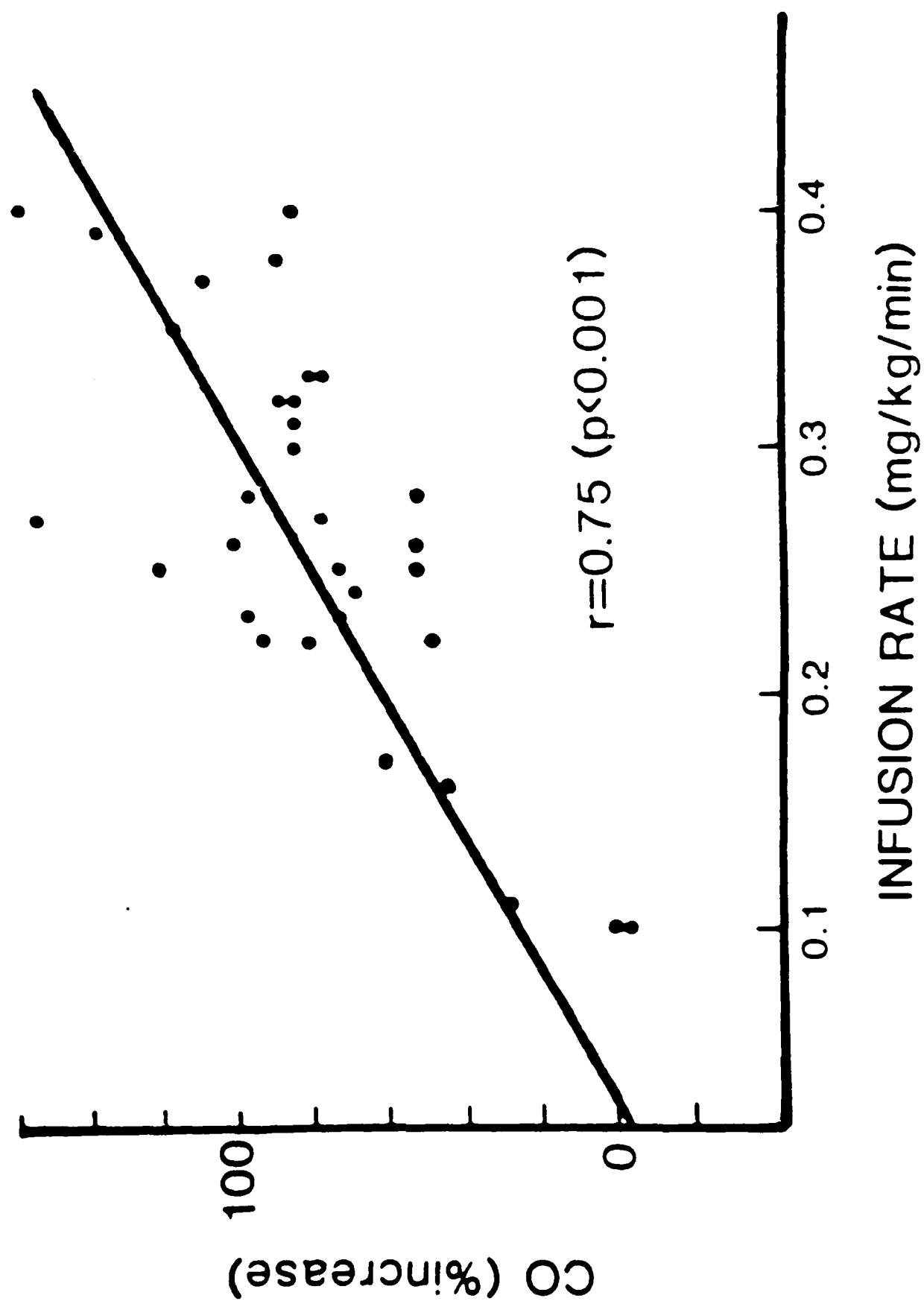
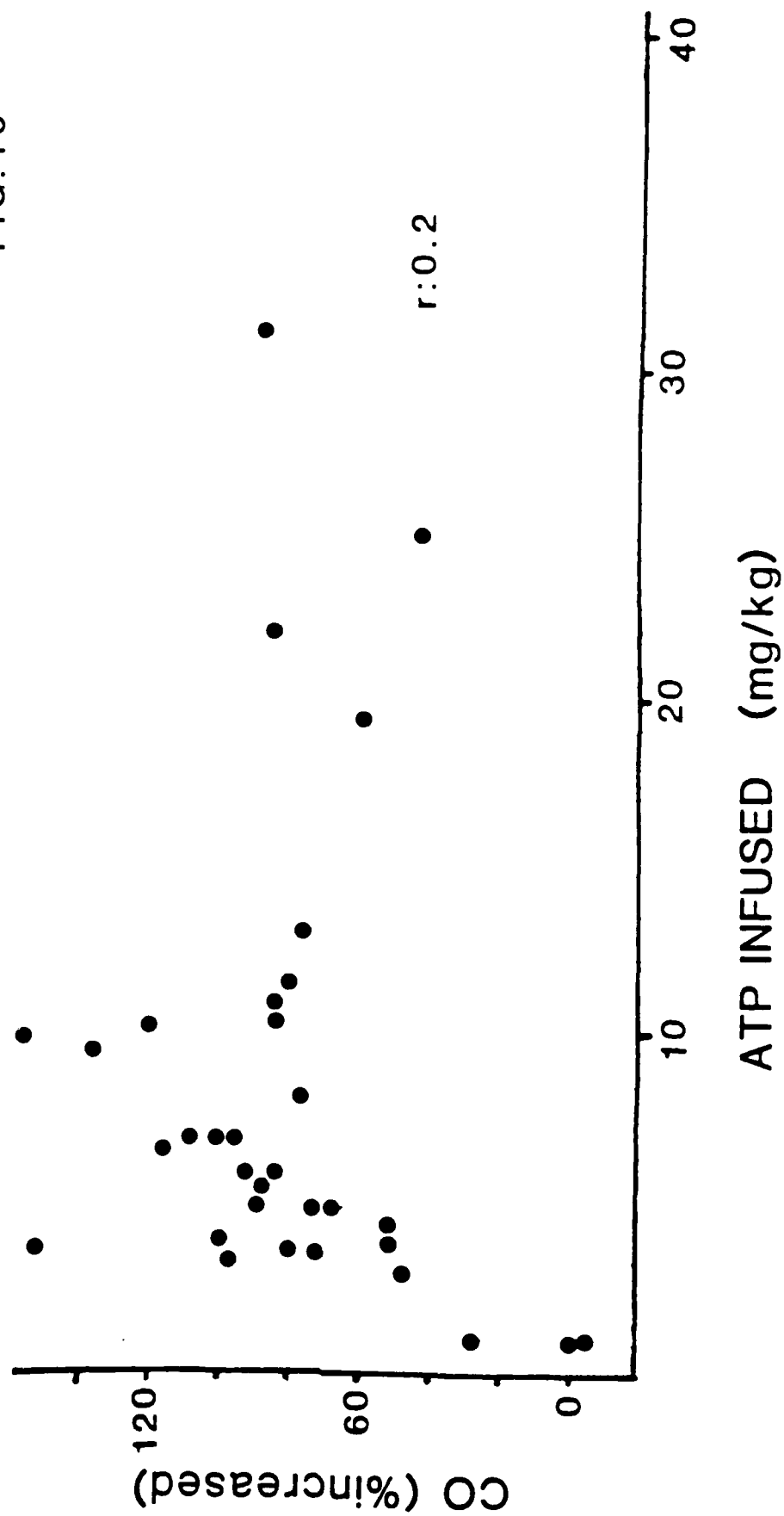
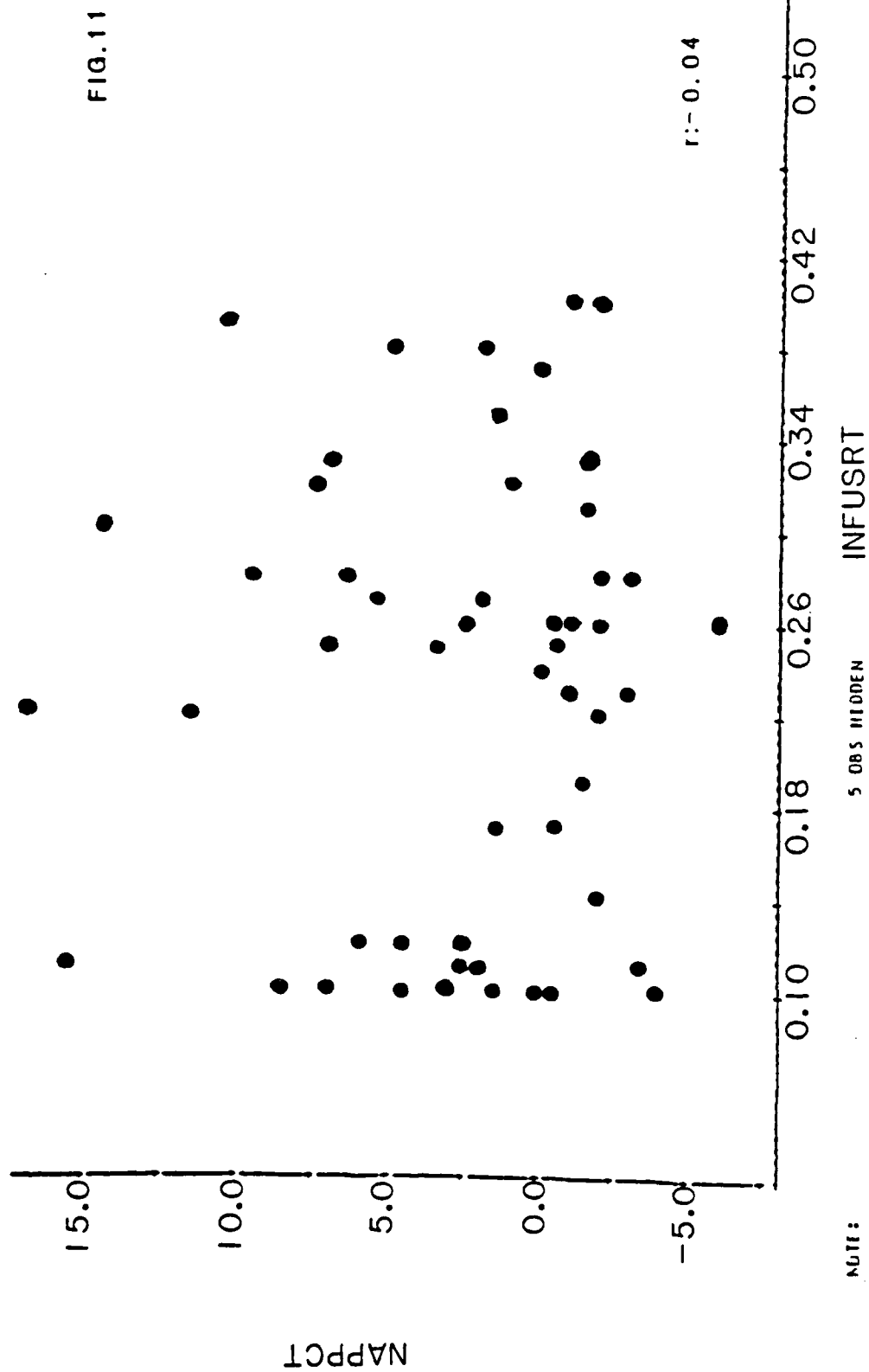
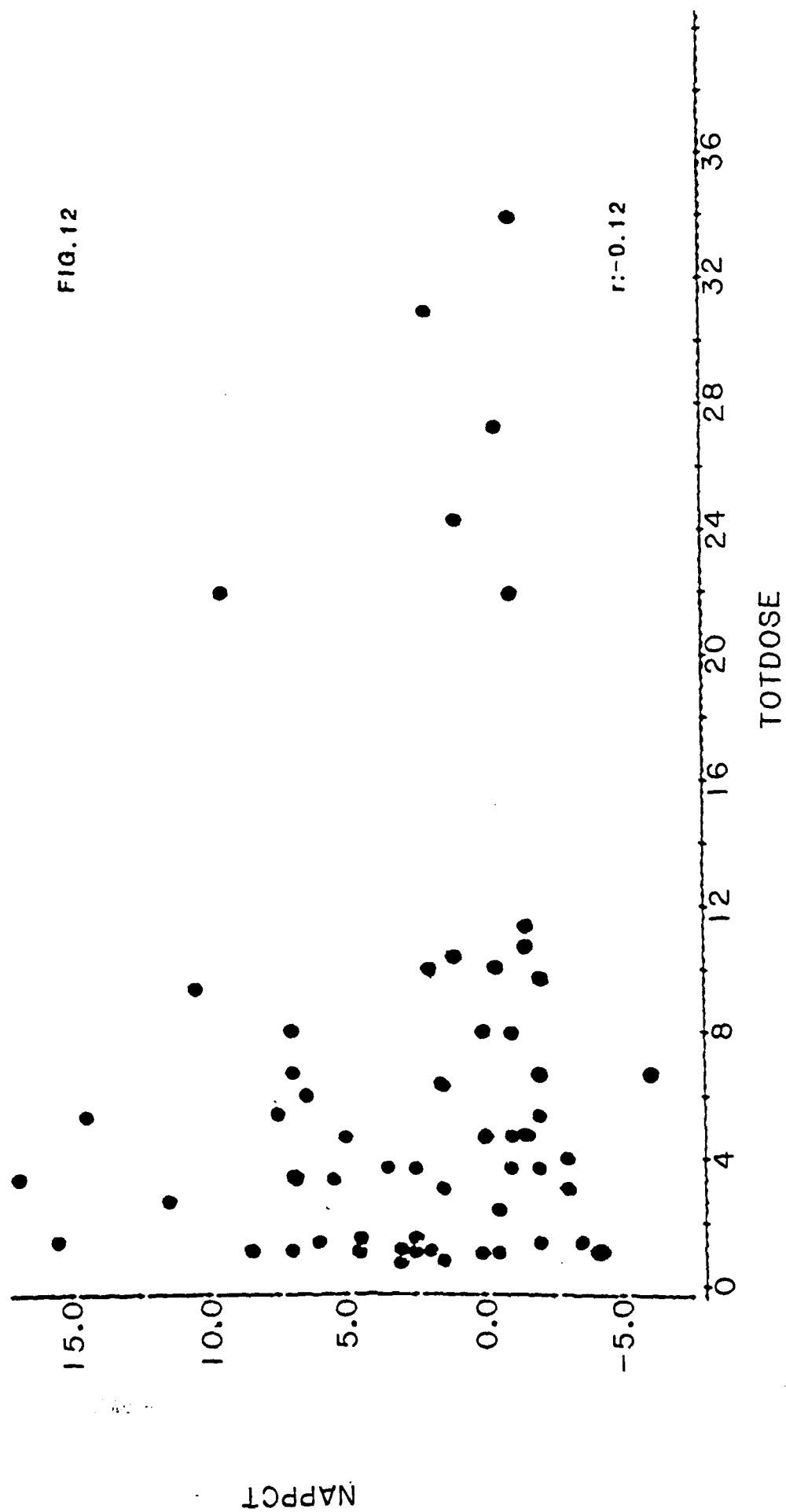


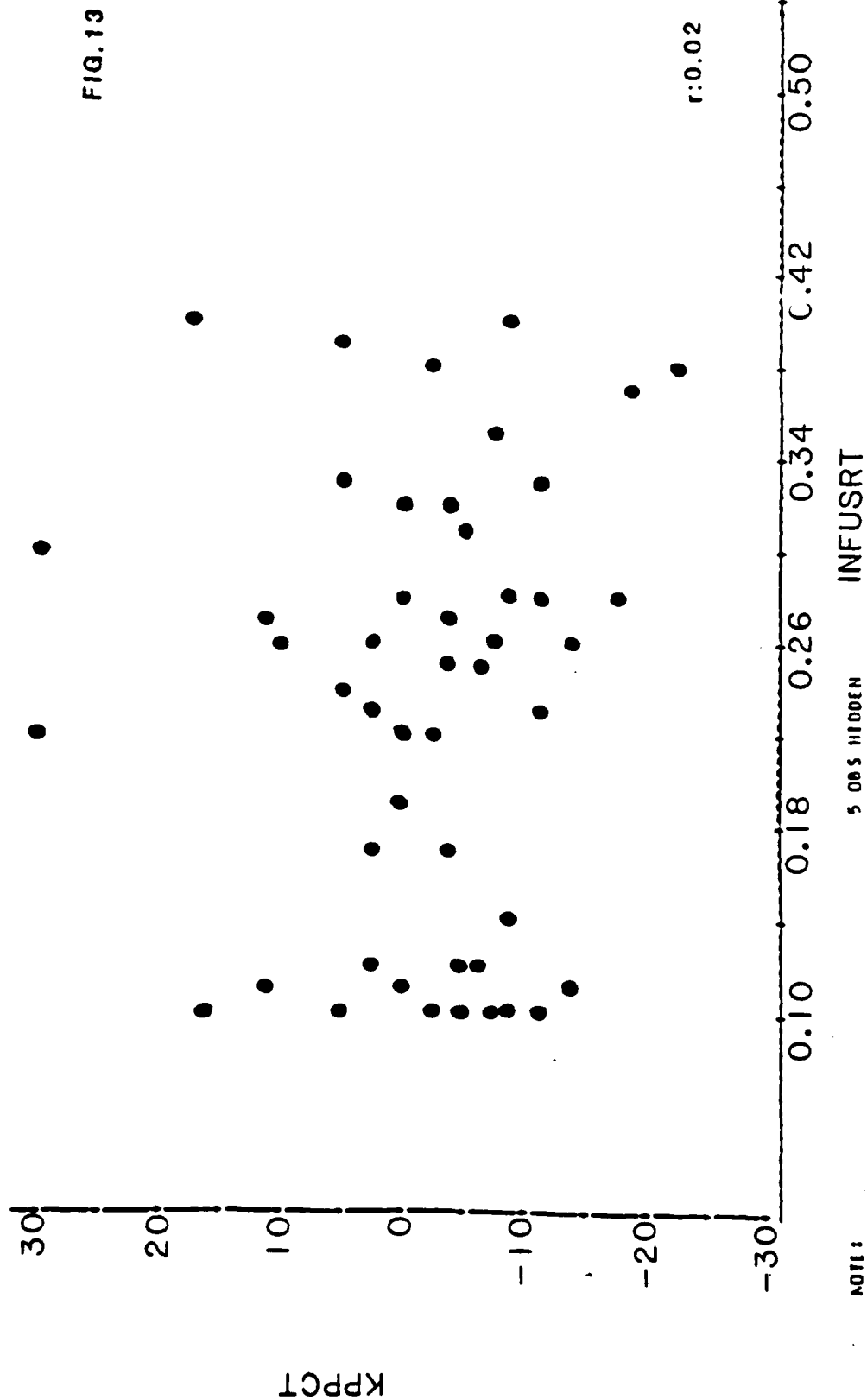


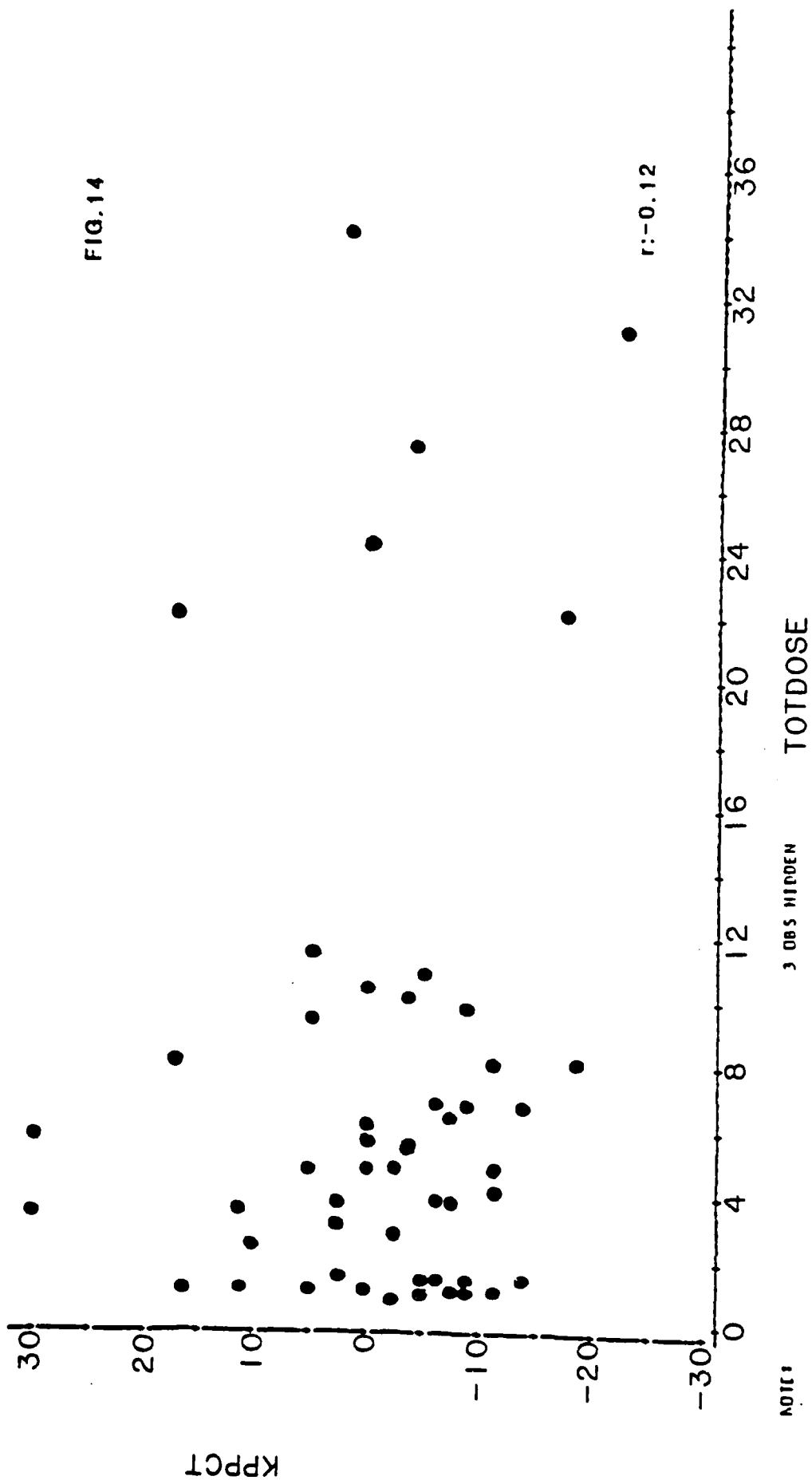
FIG. 10

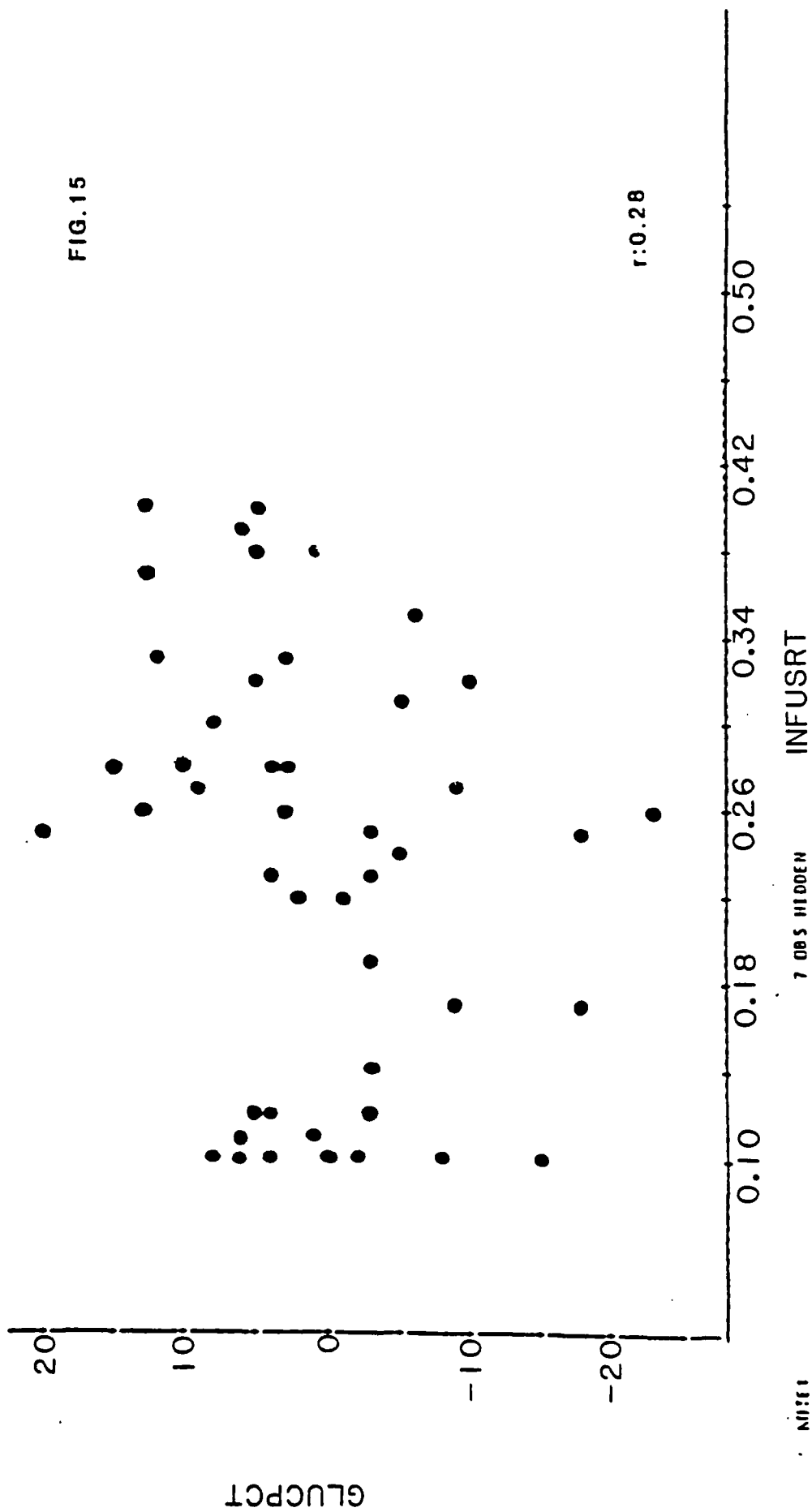












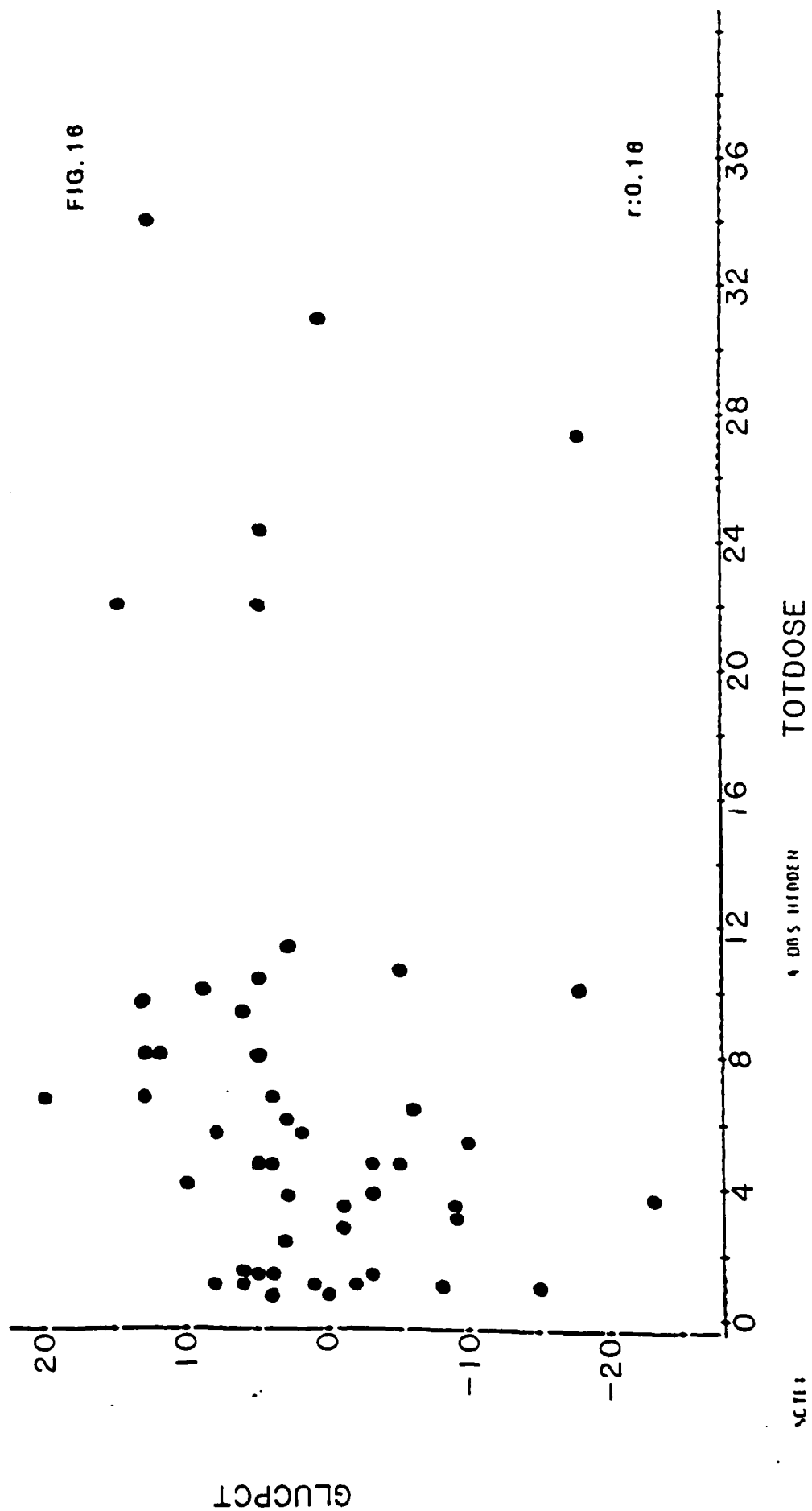
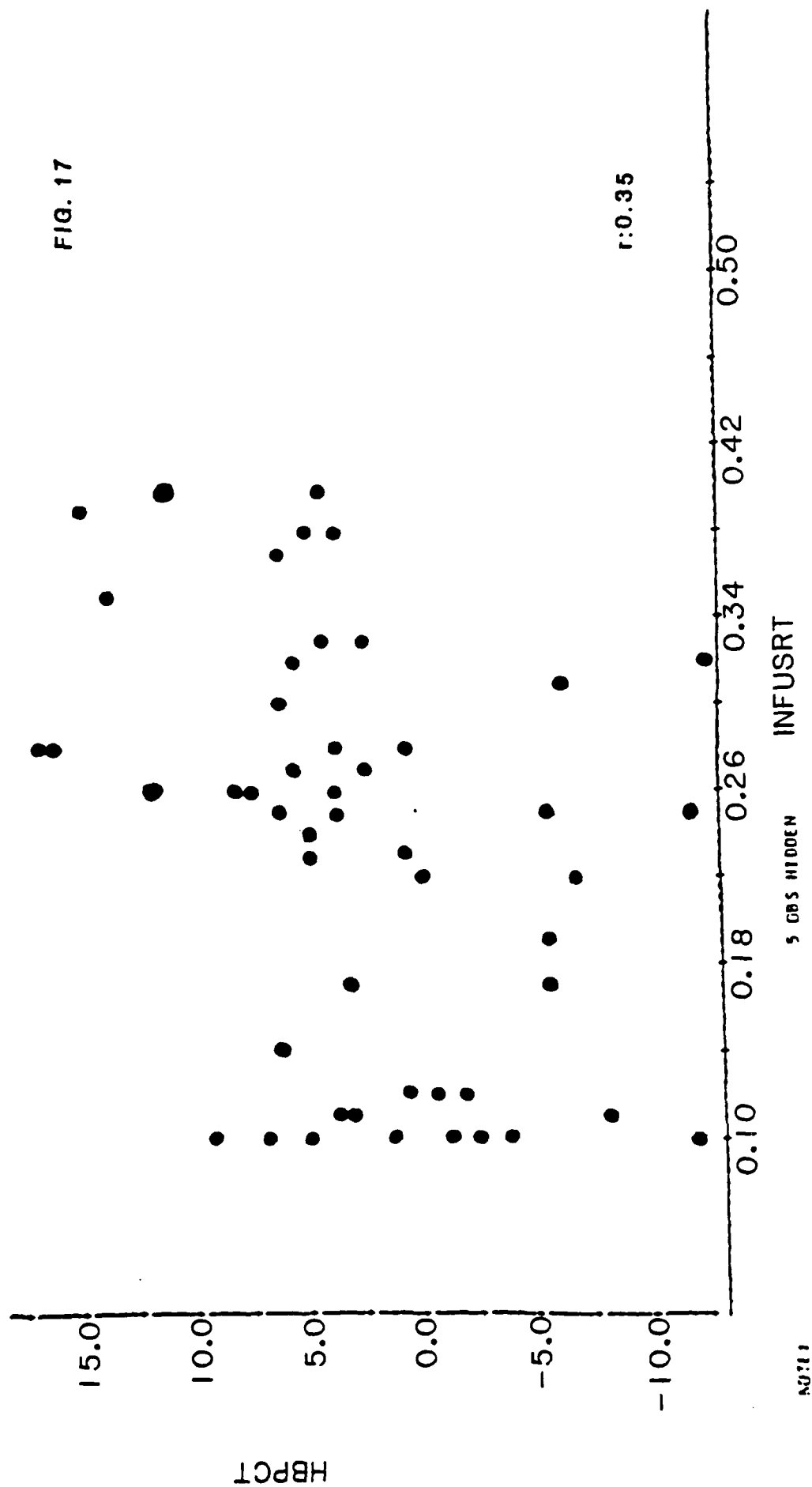


FIG. 17





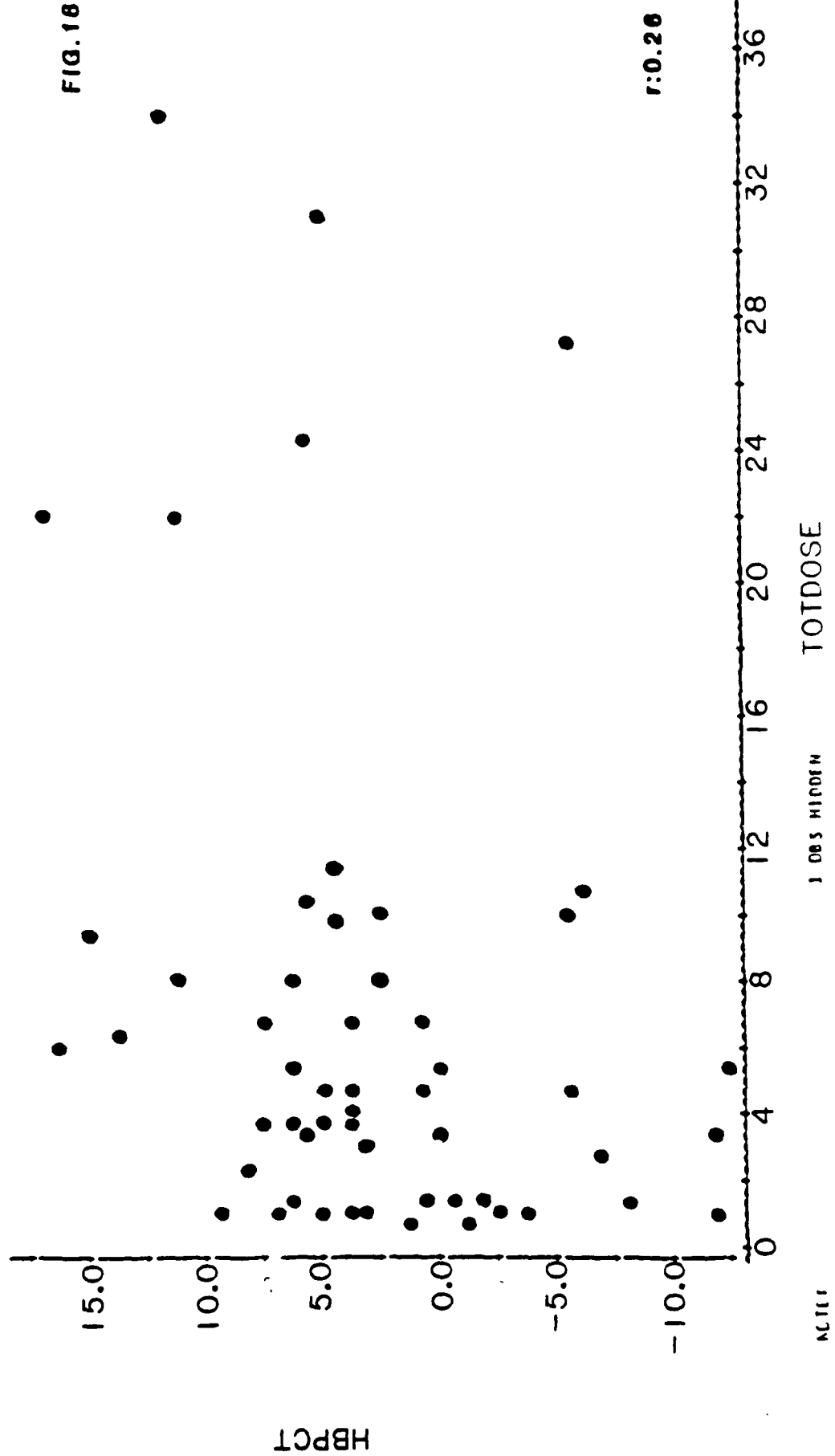


TABLE I. EFFECT OF ATP-MgCl<sub>2</sub> ON CARDIAC OUTPUT AND PERIPHERAL VASCULAR RESISTANCE.

ATP-MgCl <sub>2</sub> Infusion rate (mg/kg/min)	<u>P A R A M E T E R S.</u>				
	<u>Heart Rate</u>	<u>Mean BP</u>	<u>Cardiac Output</u>	<u>SVI</u>	<u>SVR</u>
0 - 0.01 (Group 1)	74.3 $\pm$ 1.6	92.3 $\pm$ 1.0	7.7 $\pm$ 0.4 0	0.057 $\pm$ 0.002	12.2 $\pm$ 0.53
0.10 - 0.19 (Group 2)	72.3 $\pm$ 3.2	91.6 $\pm$ 2.0	9.0 $\pm$ 1.0	0.062 $\pm$ 0.004	11.5 $\pm$ 1.18
0.22 - 0.28 (Group 3)	97.7 $\pm$ 2.9***	91.1 $\pm$ 1.8	12.8 $\pm$ 0.6***	0.068 $\pm$ 0.003**	7.3 $\pm$ 0.72**
0.31 - 0.40 (Group 4)	107.3 $\pm$ 3.6***	93.6 $\pm$ 2.3	13.6 $\pm$ 0.7***	0.065 $\pm$ 0.003*	6.8 $\pm$ 0.83**

\*\*\*p < 0.0001 compared to Group 1

\*\*p < 0.004 compared to Group 1

\*p < 0.05 compared to Group 1

Cardiac output and peripheral vascular resistance measurements in each of the 5 volunteers at 4 separate occasions were performed in the absence and presence of various rates of ATP-MgCl<sub>2</sub> infusion. Values are mean  $\pm$  S.E. BP = Blood pressure; SVI = Stroke Volume Index; SVR = systemic vascular resistance. Statistical analysis were performed with ANOVA and co-efficients of correlation.

TABLE II. EFFECT OF ATP-MgCl<sub>2</sub> INFUSION ON SERUM GOT AND GPT LEVELS (KARMAN UNITS/MT).

<u>3rd ATP-MgCl<sub>2</sub> INFUSION SERIES</u>						
		Volunteer #				
		1	2	3	4	5
Pre-ATP-MgCl <sub>2</sub> Infusion	GOT	12.19	14.38	15.37	29.90	16.25
	GPT	11.66	5.43	5.83	26.0	9.75
<u>ATP - MgCl<sub>2</sub> INFUSION RATE</u>						
		0.37mg/kg/min	0.37mg/kg/min	0.37mg/kg/min	0.33mg/kg/min	0.4mg/kg/min
During ATP-MgCl <sub>2</sub> Infusion	GOT	14.84	14.31	10.07	28.60	17.55
	GPT	14.31	5.83	6.36	24.70	7.80
7 days Post ATP-MgCl <sub>2</sub> Infusion	GOT	18.02	14.30	16.90	26.40	15.85
	GPT	12.72	5.85	7.15	25.30	8.82
<u>4th ATP-MgCl<sub>2</sub> INFUSION SERIES</u>						
		Volunteer #				
		1	2	3	4	5
Pre-ATP-MgCl <sub>2</sub> infusion	GOT	12.50	20.82	18.62		17.40
	GPT	4.00	6.25	5.62		10.02
<u>ATP - MgCl<sub>2</sub> INFUSION RATE</u>						
		0.23mg/kg/min	0.37mg/kg/min	0.32mg/kg/min		0.2mg/kg/min
During ATP-MgCl <sub>2</sub> Infusion	GOT	16.90	18.00	17.50	SAMPLES	14.50
	GPT	7.80	5.62	5.00	HEMOLYZED	10.02
7 days Post ATP-MgCl <sub>2</sub> Infusion	GOT	13.80	17.60	16.85		17.65
	GPT	8.01	5.75	5.35		10.80

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